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Genetic Species Markers and Population Structure in Alaskan Coregonid Fishes

Final Report for Study 05-702

Jeffrey B. Olsen¹, Ora Schlei¹, Randy Brown², Steve J. Miller³, Ken Harper⁴, and John K. Wenburg¹

¹Conservation Genetics Laboratory
U.S. Fish and Wildlife Service
1011 East Tudor Road, Anchorage, Alaska 99503

²Fairbanks Fish and Wildlife Field Office
U.S. Fish and Wildlife Service
101 12th Ave., Room 222, Fairbanks, Alaska, 99701

³Kenai Fish and Wildlife Field Office
U.S. Fish and Wildlife Service
PO Box 346, Bethel, AK, 99559

⁴Kenai Fish and Wildlife Field Office
U.S. Fish and Wildlife Service
P.O. Box 1670, Kenai, Alaska 99611

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Investigator(s)/Affiliation(s): Jeffrey B. Olsen, Ora Schlei and John K. Wenburg, Conservation Genetics Laboratory, USFWS; Ken Harper and Steve Miller, Kenai Fish and Wildlife Field Office, USFW, USFWS; Randy Brown, Fairbanks Fish and Wildlife Field Office, USFWS.

Geographic Area: Inter-regional

Information Type: Stock Status/Trends

Issue(s) Addressed: This project addresses two issues. First, there are no estimates of population structure of whitefish. Current management for whitefish in Alaska is based on a ‘whitefish group’ and does not differentiate populations or species. Information on the spatial scale and degree of population structure would assist managers in assessing abundance, distribution, and movement patterns and in developing sound harvest strategies. Second, there are at least eight whitefish species in Alaska and they are often difficult to identify from morphometric characteristics, particularly as juveniles. A genetic method to distinguish between species would allow us to 1) verify the species status of individual samples; 2) assess the extent and importance of hybridization between species; and 3) aid in the development of a reliable field identification key for adults and juveniles.

Study Cost: \$60,376

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Abstract: We used 14 microsatellite loci to examine population structure in humpback whitefish (*Coregonus pidschian*) from 10 locations in five regions of Alaska: Arctic, Kotzebue Sound, Yukon River, Kuskokwim River, southcentral Alaska. The results revealed significant population structure at broad (across Alaska) and fine (within the Yukon River) scales that is organized spatially at three hierarchical levels. In order of descending relative diversity these three levels are: Alaska (Arctic, western Alaska, southcentral Alaska); western Alaska (Kotzebue Sound, Yukon River, Kuskokwim River); Yukon River (Koyukuk River/mainstem at Rapids, Tanana River). These results support a conservation strategy that recognizes three spatially distinct major population groups (Arctic, western Alaska, southcentral Alaska) as the primary source of diversity but also recognizes significant population structure occurs at medium and fine-scales like western Alaska and the Yukon River. We also developed a genetic tool for identification of whitefish species in Alaska using a step-wise restriction-fragment length polymorphism assay of the cytochrome oxidase subunit I gene of mtDNA. This assay provides identification of six species and one species pair.

Key Words: Whitefish, humpback whitefish, species marker, Alaska, RFLP, microsatellites, genetics.

Project Data: Description – Data for this study consist of biological collections (fin tissue and DNA samples) and information (date and location of samples, microsatellite genotype of humpback whitefish samples, cytochrome oxidase I RFLP haplotypes for whitefish species samples). Format – Fin tissue samples stored in 95% ethanol. Sampling and genetic data are stored in a Microsoft Excel spreadsheet and Access database. Custodian(s) – U.S. Fish and Wildlife Service, Conservation Genetics Laboratory, 1011 East Tudor Road, Anchorage, Alaska 99503. Availability – Access to biological samples and data is available upon request to the custodian(s).

Report Availability: Please contact the author(s) to obtain a copy of this report.

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INTRODUCTION

There are eight recognized coregonine species in Alaska; inconnu (*Stenodus leucichthys*), round whitefish (*Prosopium cylindraceum*), pygmy whitefish (*P. coulteri*), broad whitefish (*Coregonus nasus*), Arctic cisco (*C. autumnalis*), Bering cisco (*C. laurettae*), humpback whitefish (*C. pidschian*), least cisco (*C. sardinella*). In addition, two other species, lake whitefish (*C. clupeaformis*) and Alaska whitefish (*C. nelsonii*), occur in Alaska but Alt (1979) recommends referring to them as humpback whitefish because the three species are not sufficiently distinct. Because of the difficulty distinguishing some species apart from others based on external characteristics, some species groups are combined for harvest reporting or management actions. Additionally, species identification based on external characteristics is almost always more challenging with juvenile than mature fish. Finally, there is limited information on the basic biology of these species including abundance, distribution, migratory behavior, spawning location, and population structure. Such information is particularly crucial for whitefish because they are harvested throughout the year and at different phases in their migration. Little is known about the extent to which these harvests are impacting whitefish populations and how these impacts may vary depending upon location and time of year.

Whitefish are an important target for subsistence fishing because they are available to rural Alaskan communities during much of the year. Whitefish also provide a subsistence alternative to salmon during periods of low salmon abundance, such as occurred in western Alaska in the late 1990s and early 2000s. In addition to subsistence, a commercial fishery was recently established for whitefish in the lower Yukon River in western Alaska (Brown et al. 2007). The increasing profile of whitefish as a subsistence and commercial species has prompted users to

express concern about the status of whitefish in Alaska (OSM 2005). Much of the subsistence harvest of whitefish occurs on federal land in five geographic regions of Alaska: Arctic, Kotzebue Sound, Yukon River, Kuskokwim River, and Southcentral (Figure 1). In order to address the concern of users, and to assist state and federal fishery managers, we address two important questions regarding whitefish: 1) at what spatial scale does genetic population structure occur?, and 2) can genetic markers be used to distinguish species?

To investigate the first question, we examine population structure in a single species, the humpback whitefish. We focus on this species because it is common in subsistence fisheries and adequate samples are available. The geographic range of humpback whitefish extends along the coast of the Beaufort and Chuckchi Seas including the Colville, Canning and Selawik rivers in northern Alaska to the lower Yukon and Kuskokwim rivers in western Alaska (Figure 1). It is believed that there are relatively few humpback whitefish spawning areas, even in large systems like the Yukon River. Therefore we examine the hypothesis of panmixia (no population structure) at both broad and fine spatial scales and address two objectives. First, we assess broad-scale population structure by examining humpback whitefish aggregations from five geographic regions of Alaska (Figure 1). Second, we assess fine-scale population structure by examining humpback whitefish aggregations within the Yukon River.

To address the second question, we investigate the development of a genetic method to distinguish between species that allows verification of the species status for individual samples, which could be used to develop a reliable field identification key for adults and juveniles. We examine the gene cytochrome oxidase subunit I (COI) from mitochondrial DNA (mtDNA), which has proven useful for identifying individuals of other related fish species (Hebert et al. 2003; Ward et al. 2005; Spies et al. 2006), in an attempt to develop diagnostic genetic markers to

discriminate among eight Alaskan whitefish species: inconnu, broad whitefish , humpback whitefish, Arctic cisco, Bering cisco, least cisco, round whitefish, and pygmy whitefish.

METHODS

Sample Collection

Samples of fin tissue were collected from thirteen humpback whitefish aggregations representing ten locations in five regions of Alaska (Figure 1, Table 1). All samples were from adults and were collected between 1998 and 2004 and archived in 95% ethanol at the U.S. Fish and Wildlife Service Conservation Genetics Laboratory (CGL). Multi-year samples were taken at two locations in the Yukon River (the mainstem at Rapids and the Kanuti River) and in Kotzebue Sound to test for temporal genetic variation.

Four to six individuals from each of eight whitefish species were used develop and test a species identification assay (Table 2).

Objectives 1 and 2

Genotyping

A total of 24 microsatellite loci were screened in 30 individuals for potential use in humpback whitefish: *BWF1*, *BWF2* (Patton et al. 1997); *C2-157* (Turgeon et al. 1999); *C4-157* (Turgeon 2000); *Cocl-14* (Bernatchez pers com.); *Cocl-Lav4*, *Cocl-Lav6*, *Cocl-Lav10*, *Cocl-Lav18*, *Cocl-Lav22*, *Cocl-Lav23*, *Cocl-Lav28*, *Cocl-Lav32*, *Cocl-Lav41*, *Cocl-Lav45*, *Cocl-Lav49*, *Cocl-Lav52*, *Cocl-Lav61*, *Cocl-Lav68*, *Cocl-Lav224* (Rogers et al. 2004); *PuPuPy* (Morris et al. 1996); *Sfo8*, *Sfo18*, *Sfo23* (Angers et al. 1995). Of these loci, 13 were used to genotype all putative populations (Table 3). The PCR primers for *Sfo8* produced two groups of alleles. These

alleles were determined to represent unlinked (independently segregating) loci and were treated as separate microsatellites (*Sfo8a*, *Sfo8b*) for analysis, for a total of 14 independent loci

Microsatellite genotyping consisted of first isolating total genomic DNA from fin tissue using the Qiagen 96-well DNeasy KitTM. Amplification of these 14 loci was carried out in 10ul volume PCRs: approximately 30ng DNA, 1.5 mM MgCl₂, 8mM dNTPs, 0.5 U *Taq* DNA polymerase (Promega), 0.4uM unlabeled/labeled forward primer, and 0.4uM reverse primer, using an MJResearchTM DNA EngineTM PCT-200. Cycling conditions were 2 min at 92°; 30 cycles of 15 sec at 92°, 15 sec at 54-60° (locus-specific annealing temperature), and 30 sec at 72°; with a final extension for 10 min at 72°. Microsatellites were separated and visualized on 64-well denaturing polyacrylamide gels using a Li-Cor IR² scanner and scored with Li-Cor SagaTM GT version 3.0 software (Lincoln, NE). Li-Cor 50-350 or 50-500 base size standards were loaded in the first and last lanes and at intervals of 14 lanes or less across each gel. The fragment lengths (size of microsatellite alleles) were estimated by interpolation using the computer program Saga GT ver 3.0 (Li-Cor, Lincoln, NE). An allele ladder (multiple individuals combined in a single sample) was created for each locus and was run every sixteen lanes to ensure consistency of the fragment length estimates among gels. All scores were verified by two independent researchers, with any discrepancies being resolved by re-running the samples in question and repeating the process until scores matched.

Intra-population genetic diversity

Estimates of allele frequency, allele richness (A_r), and observed and expected heterozygosity (H_o , H_e) were computed for each locus and aggregation using the computer program FSTAT version 2.9.3 (Goudet 2001). A randomization test of the statistic f (inbreeding coefficient) was used to test for conformity to Hardy-Weinberg equilibrium (HWE) for each locus and aggregation

combination. A randomization test of the log-likelihood G -statistic was used to test for genotypic disequilibrium among locus pairs over all aggregations. These tests were performed using FSTAT and the threshold for statistical significance ($\alpha = 0.05$) was corrected for simultaneous tests using the sequential Bonferroni method (Rice 1989). Two corrections were used for the HWE test to evaluate each aggregation over multiple loci and each locus over multiple aggregations.

Inter-population genetic diversity

A G -test of genotypic frequency homogeneity was used to test for significant genetic divergence among all aggregations and between pairs of aggregations. Estimates of the relative degree of genetic divergence among aggregations (F_{ST}) was computed according to Weir and Cockerham (1984) for all locations (Objective 1), for Yukon River locations (Objective 2), and for all pairs of aggregations using FSTAT. Analysis of Molecular Variation (AMOVA) was used to examine hierarchical gene diversity by quantifying between-region (F_{RT}) and within-region (F_{SR}) components of genetic divergence. A randomization test was used to assess statistical significance of each value. AMOVA was performed on all aggregations (Objective 1) and on Yukon River samples (Objective 2) using ARLEQUIN version 3.01 (Excoffier et al. 2005).

Principle Component Analysis (PCA) was used to visually assess the distribution of genetic variation among aggregations. The computer program PCAGEN version 1.2 (<http://www2.unil.ch/popgen/softwares/pcagen.htm>) was used to estimate the proportion of variation among aggregations along axes in multidimensional space. The statistical significance of individual axes was determined by performing 1,000 randomizations of genotypes using the program's randomization function as described by Lugon-Moulin and Hausser (2002).

Objective 3

Develop and test species identification assay

A species identification assay for whitefish was developed using restriction fragment length polymorphism (RFLP) in the COI gene of mtDNA (Ward et al. 2005). This process involved two steps and was conducted by researchers at the University of Laval, Quebec, Canada. First, the COI gene was sequenced in at least two individuals from each species. Second, the COI-DNA sequence for each species was screened using the freeware Sequence Analysis ver. 1.5.9 (<http://informagen.com/SA/>) to determine how many enzyme recognition sequences were present within the gene and the length (in base pairs, bp) of the restriction fragments. These results were compared across species to determine if diagnostic fragment length polymorphisms were present.

Testing of the RFLP assay was performed at the CGL. This process also involved two steps. First, the genetic assay was tested in four to six individuals (Table 3) from each species to verify the restriction fragments could be clearly identified using standard agarose gel electrophoresis and to verify the assay was robust and repeatable. For this step, the COI gene was amplified by PCR using the protocol and primers FishF1 and FishR1 from Ward et al. (2005). Restriction digests of the COI gene were performed for each enzyme in the diagnostic suite according to the manufacturer's specifications (New England BioLabs, Beverly, Maine). The product from each digest was visualized following electrophoresis on 2.5% high resolution agarose gel stained with ethidium bromide. A 100-1000 bp ladder was included in the first, middle, and last lane of each gel to aid in identifying fragments.

The second step was a blind test of the genetic assay. Fifty individuals representing all species were provided unlabeled and unordered to the researcher conducting the assay. This sample did not necessarily contain the same individuals as in step 1. The assay was conducted as described

above and each individual was assigned a species based on the results of the restriction digests. The species assignments from the assay were compared to the known identities in order to assess the accuracy of the test.

RESULTS

Objectives 1 and 2

Intra-population genetic diversity

The mean estimates of allele richness (A_r) and expected heterozygosity (H_e) were 6.7 and 0.65 when average over all loci and aggregations. The Paxson Lake sample from southcentral Alaska exhibited the lowest estimates of A_r and H_e (4.3 and 0.52) when averaged over all loci (Table 3), and the lowest estimates of A_r and H_e at eight and seven individual loci, respectively. Of the remaining aggregations, the two from the upper Tanana River in the Yukon River region (Fish Lake and Moose Creek) exhibited lowest mean estimates of A_r and H_e . The highest mean estimates of A_r and H_e per aggregation were 7.8 (Selawik River Delta, 2004) and 0.71 (Canning River).

Multiple randomization tests of conformity to HWE for each locus and aggregation revealed 13 tests where the P -value for the test statistic f was below 0.05 because of a deficit of heterozygotes (Table 3). Five tests were associated with a single locus (*Cocl-Lav45*) and the P -values for two of these tests were judged significant when the α -level was adjusted for multiple aggregations. Therefore, this locus was removed from the data set. Of the remaining eight tests, three loci (*BWF2*, *Cocl-Lav4*, *Cocl-Lav68*) were associated with a single aggregation (the Yukon River mainstem at Rapids, 2004), however, the P -values were not judged significant when the α -level was adjusted for multiple loci.

Randomization tests for genotypic disequilibrium revealed 10 locus pairs with a P -value for the G -statistic below 0.05. These results were not judged significant when the α -level was adjusted for 78 pairwise tests.

Inter-population genetic diversity

The G -test of genotypic frequency homogeneity indicated significant genetic differentiation over all 13 aggregations and for 61 of the 78 pairwise comparisons ($P < 0.001$, Table 4). Most (14/17) of the non-significant pairwise tests involved pairs of aggregations within the Yukon River. In fact, within the Yukon River aggregations, only 7 out of 21 pairwise tests were significant, and all of those included one of the two Tanana aggregations (i.e., Fish Lake or Moose Creek). The G -test provided no evidence of inter-annual variation in genotypic frequency at the three locations for which temporal replicates were included (Selawik River Delta, Yukon River mainstem at Rapids, Kanuti River).

The estimate of F_{ST} over all 13 aggregations was 0.067 and was significantly greater than zero ($P < 0.010$). The estimate of F_{ST} over all seven Yukon River aggregations was 0.012 and was significantly greater than zero ($P < 0.010$). The pairwise F_{ST} estimates for the aggregations within and among the three western Alaska regions (Kotzebue Sound, Yukon River, and Kuskokwim River, Table 4) were all < 0.05 , while most of those that included the Arctic and southcentral region aggregations were greater than 0.10 (40/43, Table 4).

The AMOVA results showed that most of the broad-scale genetic variation was explained by variation among the five regions (F_{RT}) rather than variation within the regions (F_{SR} , Table 5), although both values were significantly greater than zero ($P < 0.001$).

Most of the genetic variation within the Yukon River was explained by variation among (F_{RT}) rather than within (F_{SR}) groups when the seven aggregations were grouped by mainstem, Tanana and Koyukuk (Table 5)

The first two principle components of the PCA provided a visual depiction of the trend in genetic relationships observed in the pairwise F_{ST} values (Figure 2). At a broad scale, aggregations from the three western Alaska regions were similar and formed a group distinct from aggregations in the Arctic and southcentral Alaska regions (Figure 2A). The observed pattern explained over 65% of the total genetic variation and both axes were significant ($P < 0.001$). However, at a finer scale within western Alaska, the populations formed three regional groups, and further, within the Yukon River, aggregations clustered by location with the exception of the Kanuti River, 2004 (Figure 2B). At this finer scale, the observed pattern explained over 60% of the total genetic variation and both axes were significant ($P < 0.001$).

Objective 3

Develop and test species identification assay

The COI gene of mtDNA (approximately 655 bp) was sequenced in two individuals from nine whitefish species. The sequence was screened using sequence recognition software to identify restriction enzyme cut sites. The number of enzymes with COI cut sites ranged from 116 (broad whitefish) to 143 (round whitefish) and averaged 126 (Appendices 1-8). The number of cut sites across all enzymes ranged from 250 (pygmy whitefish) to 321 (Bering cisco) and averaged 291. The DNA fragments produced by four enzymes, *RsaI*, *HaeIII*, *BseRI*, *BsmI*, appeared to be useful for species identification when combined in a step-wise RFLP assay (Table 6). First, *RsaI* isolated pygmy whitefish and inconnu. The remaining species formed a single group (*RsaI*-3) that lacked the *RsaI* cut site. Second, *HaeIII* isolated round whitefish and least cisco (step 2).

The remaining five species formed two groups with different fragment lengths. Next, *BseRI* (step 3a) isolated arctic and Bering cisco (group *HaeIII-3*). Finally, *BsmI* (step 3b) isolated broad and humpback whitefish. Forty-eight of 49 individuals were correctly assigned to species in a blind test of this assay. One putative Bering cisco was twice identified as an arctic cisco in separate assays following independent DNA extraction.

DISCUSSION

Objectives 1 and 2

Intra-population diversity in Alaskan humpback whitefish

The low level of variation within the Paxson Lake sample from southcentral Alaska, as compared to the other 12 aggregations, is notable. This result may reflect population history as humpback whitefish in southcentral Alaska populations are believed to have been colonized from the Tanana River in the Yukon River drainage during retreat of the Pleistocene ice (Lindsey and McPhail 1986). Such an event could have resulted in a loss of diversity (founder effect). The relative low diversity in the Paxson Lake sample may also reflect greater isolation from neighboring aggregations. Humpback whitefish are located in other areas of the Copper River drainage including Crosswind Lake and the Slana River in the upper drainage and McKinley and Martin lakes in the lower drainage. Additional sampling is needed to examine gene flow and assess if these other aggregations exhibit a similar level of intra-population diversity.

We failed to reject the null hypothesis of Hardy-Weinberg equilibrium (HWE) for each sampled aggregation. Therefore, we cannot conclude that any aggregation represents an admixture of populations. It should be noted, however, that admixtures of weakly differentiated populations, such as we found in the Yukon River may not deviate significantly from HWE. One possible

example of this is the aggregation sample from the Yukon River mainstem at Rapids in 2004. The P -value for four of 14 loci for this sample was below 0.05 although they were not significant after correcting for multiple tests. While spawning may occur in the mainstem, it may also be that multiple weakly differentiated populations congregate in this area while traveling to spawn in tributaries like the Tanana and Koyukuk, or other unsampled locations.

Broad-scale population structure in Alaskan humpback whitefish

This study provided the first indication of the level and spatial distribution of population structure in Alaskan humpback whitefish. The overall value of F_{ST} (0.067) suggests moderate population structure, however, substantial spatial heterogeneity exists in the distribution of genetic diversity. Most of the inter-population diversity is due to regional-level variation as indicated by AMOVA. The PCA and pairwise F_{ST} values indicate this broad-scale variation is primarily explained by three major regional groups. The western Alaska aggregations (from the Kotzebue Sound, Yukon and Kuskokwim River regions) form a single group with a relatively modest level of divergence among the three regions (mean pairwise $F_{ST} = 0.029$). In contrast, the level of population divergence among aggregations from western Alaska and the Arctic and southcentral regions is nearly five times greater (mean pairwise $F_{ST} = 0.140$) and generally indicative of highly fragmented or isolated populations (Frankham et al. 2002).

A number of factors may explain this dichotomy in the level of population divergence including historical events and contemporary factors such as gene flow and genetic drift. Regarding population history, the relatively high degree of divergence of the three population groups is consistent with the relatively high degree of divergence between two mitochondrial DNA haplotypes in lake whitefish from the Yukon River and other areas of Alaska (Bernatchez and Dodson 1991). They assign the two haplotypes to a single glacial refugium (Beringia), however,

they acknowledge the high divergence may reflect a contact zone between different assemblages isolated within Beringia or expansion from Eurasia (Bernatchez and Dodson 1991).

Interestingly, one of their two haplotypes was not found in southcentral Alaska. Bernatchez and Dodson (1991) also identified a third haplotype in Northwestern Canada and eastward they assigned to an eastern glacial refugium. Because they did not sample the Arctic region in Alaska, it is not clear if this haplotype (that they did not find in the Yukon River area and southcentral Alaska) extends further west to populations in northern Alaska. However, given the geographic proximity, it seems likely that populations from northern Alaska and Northwestern Canada could share a common population history.

Regarding contemporary factors, both gene flow and genetic drift may also contribute to the dichotomous nature of regional population structure. Gene flow among humpback whitefish aggregations from western Alaska, the Arctic and southcentral Alaska is likely to be very low or non-existent given the large geographic distances, physical barriers (e.g. Alaska Peninsula) and ecological differences between the areas. In contrast, humpback whitefish from the three regions in western Alaska are anadromous and likely overlap in their coastal migrations, so it is reasonable to expect more gene flow, and therefore less divergence, among these aggregations. While this argument also applies to the two aggregations from the Arctic, more likely sources of immigrants to the Colville and Canning rivers could be aggregations from Northwestern Canada.

Significant regional-level structure does exist in western Alaska, as was evident from the PCA and pairwise F_{ST} values as well as a post-hoc AMOVA that showed inter-regional variation ($F_{RT} = 0.020$) was twice as large as intra-regional variation ($F_{SR} = 0.011$) and both values were significantly larger than zero ($P < 0.001$).

These regions appear to be the primary source of diversity for humpback whitefish in western Alaska.

Fine-scale population structure in humpback whitefish

We examined fine scale population structure by sampling five locations within the Yukon River. The overall value of F_{ST} (0.012) indicated weak but significant population structure throughout the drainage likely due to at least two distinct spawning aggregations. This level of genetic divergence is relatively small compared to that reported for other whitefish species in a similar or smaller geographic range including mountain whitefish in Montana and lake cisco in eastern Canada (Turgeon and Bernatchez 2001; Whiteley et al. 2004). This difference may reflect the influence of anadromy as both mountain whitefish and lake cisco are freshwater species with limited migration potential. Anadromy likely increases the potential for long range gene flow among humpback whitefish in a large system like the Yukon River.

The AMOVA and G-test results suggest that spawning aggregations in the Koyukuk and Tanana rivers are at least partially reproductively isolated. The mainstem aggregations, while genetically distinct from those in the Tanana River, were not significantly different from the Koyukuk River aggregations. In addition, there was no evidence of genetic variation between spatially distinct pairs of aggregations within the Tanana and Koyukuk rivers. These results suggest a prudent management strategy acknowledge at least two groups of humpback whitefish aggregations within the Yukon River, Koyukuk River/mainstem at Rapids and the Tanana River. Sampling of additional spatially distinct aggregations will be needed to determine if more complex fine-scale population structure exists in the Yukon River.

In addition to fine-scale spatial structure we tested for temporal variation within three locations. Two of the locations, the Selawik River Delta in Kotzebue Sound and the Kanuti River in the Yukon River drainage, were sampled one year apart. The third location, the mainstem of the Yukon River at Rapids, was sampled five years apart. The temporal replicates were not genetically distinct, regardless of the duration between samples. This is tentative evidence of cohesion among cohorts within spawning aggregations consistent with philopatry. This also suggests the spatial signals identified above are not likely to vary in the near future.

Summary of population structure and conservation implications

The population structure of humpback whitefish examined in this study is organized regionally at three hierarchical levels. The first level consists of three major regional aggregations that represent the greatest degree of spatial genetic diversity. These three major regions are: Arctic, western Alaska (Kotzebue Sound, Yukon River, Kuskokwim River), and southcentral Alaska. The genetic diversity among humpback whitefish from these three major regions is indicative of very limited or non-existent gene flow and may also reflect different evolutionary histories. These results suggest conservation efforts should focus first at this level. The second level consists of the three regional aggregations from western Alaska; Kotzebue Sound, Yukon River, Kuskokwim River. The genetic diversity among humpback whitefish from these three regions is modest compared to the first level, likely due to some inter-regional gene flow. Nevertheless, the inter-regional estimate of genetic diversity is significant, suggesting some reproductive isolation. The three regions appear to be the primary source of diversity and thus the starting point for conservation within western Alaska. The final and finest level consists of two groups of aggregations within the Yukon River; Koyukuk River/mainstem at Rapids and the Tanana River. The level of genetic diversity among these two groups, while less than the level of

genetic diversity among the three western Alaska regions, is nevertheless significant and warrants consideration when developing conservation guidelines.

Objective 3 – Whitefish species identification

The RFLP protocol described here provides a promising tool for identification of whitefish species in Alaska. The results indicate accurate identification is possible for at least six (pygmy whitefish, inconnu, round whitefish, least cisco, broad whitefish, humpback whitefish) of the eight species examined. We conservatively conclude the protocol can identify a Bering/arctic cisco species group, but not the two species. Further examination is necessary to determine if the single misidentified Bering cisco reflects an “arctic cisco” polymorphism in the Bering cisco COI gene. Such a result would not be surprising given that the two species were once considered part of a single species complex, *C. autumnalis* (McPhail 1966). Nonetheless, the existing genetic protocol should be useful in addressing a number of management and research related issues where other methods of species identification are unreliable and distinction between Bering and Arctic cisco is of little or no importance.

RECOMMENDATIONS

- Expand the analysis of population structure to other whitefish species of concern.
- Further examine fine-scale population structure of humpback whitefish within the Yukon River, or other regions of interest, by including other likely spawning populations as well as additional temporal replicates.
- Consider collecting voucher specimens of each species for archiving and integration with other DNA barcoding projects.

- Evaluate the potential of developing a quicker alternative to RFLP assay to reveal the species-specific sequence polymorphisms in the COI gene (such as a single nucleotide polymorphism assay).

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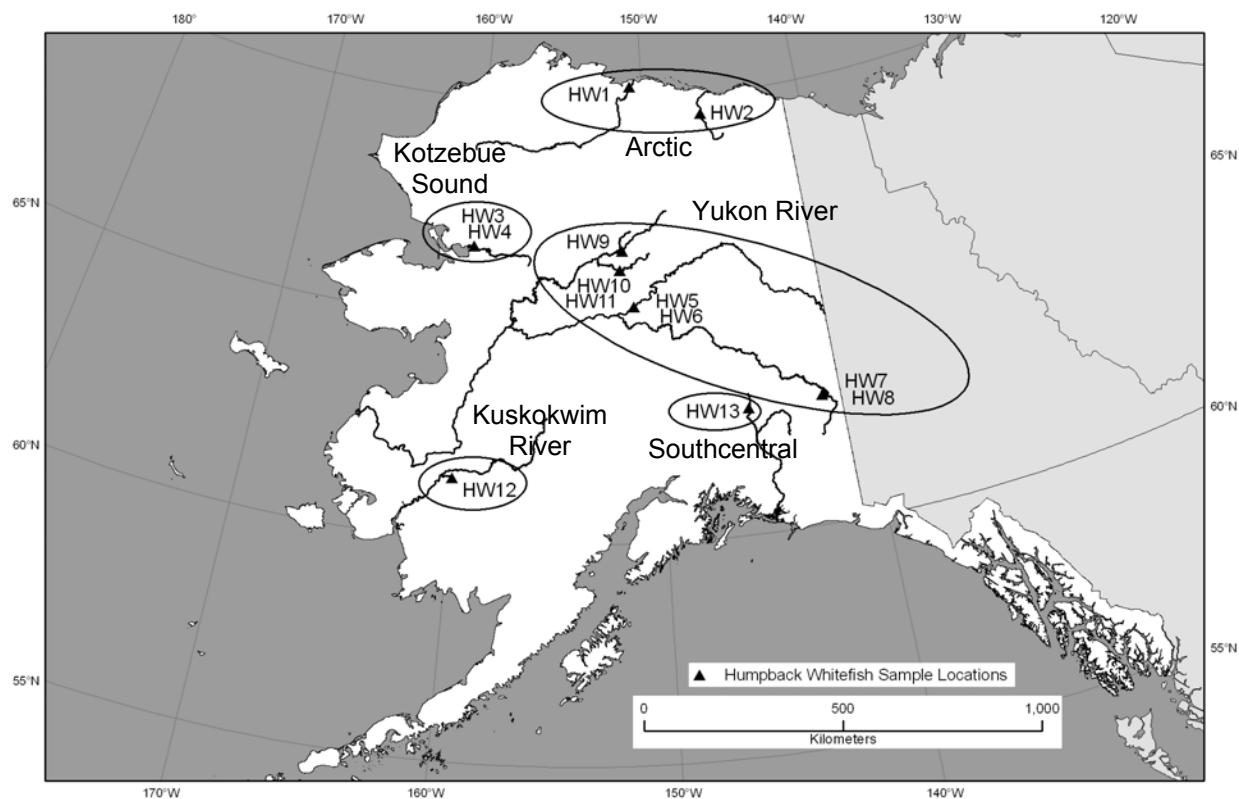


Figure 1. Location of humpback whitefish aggregations sampled from five regions in Alaska. Sample abbreviations are Colville River (HW1), Canning River (HW2), Selawik River Delta 2003 (HW3), Selawik River Delta 2004 (HW4), Yukon River mainstem at Rapids 1999 (HW5), Yukon River mainstem at Rapids 2004 (HW6), Fish Lake (HW7) Moose Creek (HW8), South Fork Koyukuk River (HW9), Kanuti River 2003 (HW10), Kanuti River 2004 (HW11), Whitefish Lake (HW12), Paxson Lake (HW13).

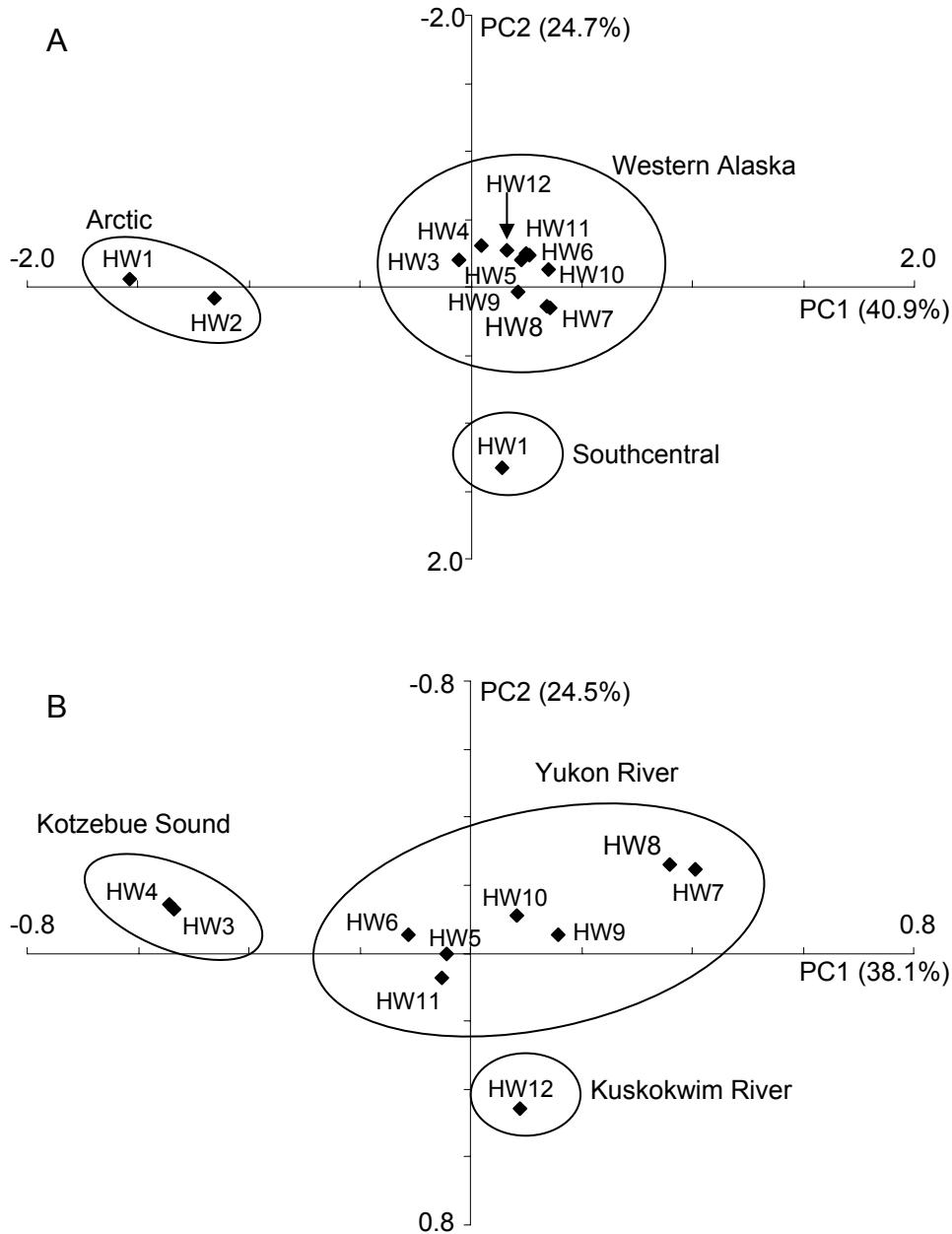


Figure 2. Principal component analysis (PCA) of allele frequency data from 13 loci in humpback whitefish. PC axes 1 and 2 capture 40.9% ($P < 0.001$) and 24.7% ($P < 0.001$) of the variation across all populations (plot A) and 38.1% ($P < 0.001$) and 24.5% ($P < 0.001$) of the variation across Western Alaska populations (plot B). Sample abbreviations are Colville River (HW1), Canning River (HW2), Selawik River Delta 2003 (HW3), Selawik River Delta 2004 (HW4), Yukon River mainstem at Rapids 1999 (HW5), Yukon River mainstem at Rapids 2004 (HW6), Fish Lake (HW7) Moose Creek (HW8), South Fork Koyukuk River (HW9), Kanuti River 2003 (HW10), Kanuti River 2004 (HW11), Whitefish Lake (HW12), Paxson Lake (HW13).

Table 1. Humpback whitefish samples used to examine population structure in Alaska.

Region Aggregation (year)	Month or Season	<i>n</i>
Arctic		
Colville River (2004)	Spring	23
Canning River (1999)	July-Aug	55
Kotzebue Sound		
Selawik River Delta (2003)	n/a	53
Selawik River Delta (2004)	Spring	50
Yukon River ^a		
Mainstem Rapids (1999)	n/a	38
Mainstem Rapids (2004)	Fall	35
Tanana River Fish Lake (1998)	n/a	55
Tanana River Moose Creek (1998)	n/a	55
Koyukuk River South Fork (2003)	August	38
Koyukuk River Kanuti River (2003)	Spring	26
Koyukuk River Kanuti River (2004)	Spring	35
Kuskokwim River		
Whitefish lake (2001)	Sept-Oct	100
Southcentral		
Copper River Paxson Lake (2004)	June	50

^aYukon River samples were used to address objectives 1 and 2.

Table 2. Samples of whitefish species used to develop and test a species identification assay.

Species	Region		n	Year
	Location			
Inconnu (<i>Stenodus leucichthys</i>)	Kotzebue Sound			
	<i>Selawik River</i>	3	2003	
	Yukon River			
	<i>Nowitna River</i>	3	2003	
Broad whitefish (<i>Coregonus nasus</i>)	Kotzebue Sound			
	<i>Selawik River</i>	3	2003	
	Yukon River			
	<i>Mainstem</i>	3	2005	
Bering cisco (<i>Coregonus laurettae</i>)	Yukon River			
	<i>Mainstem</i>		2004	
	Rampart	6	2004	
Arctic cisco (<i>Coregonus autumnalis</i>)	Arctic			
	<i>Kaktovik Lagoon</i>	6	2005	
Least cisco (<i>Coregonus sardinella</i>)	Kotzebue Sound			
	<i>Selawik River</i>	3	2003	
	Yukon River			
	<i>Koyukuk River</i>	3	2003	
Humpback whitefish (<i>Coregonus pidschian</i>)	Yukon River			
	<i>Koyukuk River</i>			
	Kanuti River	3	2004	
	<i>Nowitna River</i>	3	2003	
Round whitefish (<i>Prosopium cylindraceum</i>)	Bristol Bay			
	<i>Ugashik River</i>	6	2004	
Pygmy whitefish (<i>Prosopium coulteri</i>)	Bristol Bay			
	<i>Ugashik River</i>	3	2004	
	Montana			
	<i>Lake Bitterroot</i>	1	2003	

Table 3. Genetic diversity at 14 microsatellite loci in humpback whitefish aggregations from five regions in Alaska: n = sample size, He = expected heterozygosity, Ho = observed heterozygosity, Ar = allele richness. Region abbreviations are Arctic (Arc), Kotzebue Sound (Kot), Yukon River (Yuk), Kuskokwim River (Kus), southcentral Alaska (SC). Sample abbreviations are Colville River (HW1), Canning River (HW2), Selawik River Delta 2003 (HW3), Selawik River Delta 2004 (HW4), Yukon River mainstem at Rapids 1999 (HW5), Yukon River mainstem at Rapids 2004 (HW6), Fish Lake (HW7), Moose Creek (HW8), South Fork Koyukuk River (HW9), Kanuti River 2003 (HW10), Kanuti River 2004 (HW11), Whitefish Lake (HW12), Paxson Lake (HW13).

Locus	Region/Sample												
	Arc HW1	Arc HW2	Kot HW3	Kot HW4	Yuk HW5	Yuk HW6	Yuk HW7	Yuk HW8	Yuk HW9	Yuk HW10	Yuk HW11	Kus HW12	SC HW13
<i>BWF2</i>													
n	20	51	51	43	32	30	53	51	28	17	30	97	34
H_e	0.60	0.67	0.73	0.68	0.71	0.73	0.54	0.53	0.59	0.73	0.63	0.63	0.11
H_o	0.65	0.57	0.84	0.63	0.53	0.60	0.55	0.45	0.57	0.71	0.63	0.62	0.12
A_r	5.7	4.9	6.2	5.7	6.2	8.1	4.4	4.5	6.2	7.9	6.3	4.9	1.9
<i>C2-157</i>													
n	23	53	53	45	33	30	55	54	34	19	29	95	40
H_e	0.73	0.77	0.84	0.86	0.90	0.85	0.87	0.88	0.90	0.91	0.91	0.85	0.87
H_o	0.57	0.68	0.79	0.82	0.85	0.80	0.82	0.93	0.91	0.89	0.79	0.84	0.88
A_r	6.9	9.3	11.9	11.1	11.7	10.1	9.9	10.2	10.5	12.9	11.3	10.8	9.0
<i>Cocl-Lav4</i>													
N	23	53	52	45	33	31	55	54	33	19	30	98	39
H_e	0.60	0.63	0.73	0.68	0.65	0.55	0.61	0.66	0.63	0.46	0.61	0.47	0.66
H_o	0.57	0.68	0.73	0.64	0.64	0.42	0.60	0.65	0.64	0.53	0.47	0.48	0.59
A_r	3.7	5.0	6.3	5.4	5.4	5.3	5.8	4.9	5.1	4.5	5.7	5.4	4.8
<i>Cocl-Lav6</i>													
N	23	52	52	38	32	26	53	53	34	18	28	98	40
H_e	0.81	0.87	0.83	0.85	0.77	0.80	0.84	0.82	0.81	0.80	0.78	0.81	0.75
H_o	0.87	0.79	0.87	0.87	0.91	0.77	0.92	0.92	0.85	0.89	0.71	0.89	0.75
A_r	10.2	8.7	9.4	10.3	8.1	7.5	7.7	7.3	8.0	6.0	8.3	8.7	5.7
<i>Cocl-Lav10</i>													
n	20	47	52	43	27	28	52	54	32	16	28	85	40
H_e	0.66	0.53	0.68	0.66	0.70	0.70	0.50	0.60	0.64	0.70	0.64	0.54	0.67
H_o	0.70	0.51	0.71	0.63	0.63	0.68	0.60	0.59	0.75	0.63	0.68	0.56	0.63
A_r	4.9	5.0	5.1	4.3	5.4	4.6	3.8	4.6	3.9	4.0	4.5	5.0	4.0

Table 3. cont.

Locus	Region/Location												
	Arc HW1	Arc HW2	Kot HW3	Kot HW4	Yuk HW5	Yuk HW6	Yuk HW7	Yuk HW8	Yuk HW9	Yuk HW10	Yuk HW11	Kus HW12	SC HW13
<i>Cocl-Lav18</i>													
<i>n</i>	23	52	53	45	33	31	55	54	34	19	30	97	40
<i>H_e</i>	0.64	0.56	0.46	0.38	0.32	0.28	0.31	0.24	0.32	0.23	0.33	0.40	0.18
<i>H_o</i>	0.74	0.54	0.49	0.40	0.33	0.26	0.31	0.24	0.26	0.26	0.33	0.42	0.20
<i>A_r</i>	4.0	3.4	3.1	3.6	2.0	2.8	2.0	2.0	2.0	2.0	2.8	2.8	2.0
<i>Cocl-Lav22</i>													
<i>n</i>	21	53	52	45	32	30	55	53	34	16	26	96	37
<i>H_e</i>	0.82	0.90	0.94	0.90	0.92	0.91	0.87	0.87	0.86	0.88	0.89	0.89	0.40
<i>H_o</i>	0.81	0.94	0.98	0.89	1.00	0.93	0.87	0.92	0.82	1.00	0.88	0.89	0.35
<i>A_r</i>	9.9	12.4	14.9	13.3	12.9	12.6	11.4	12.4	10.3	9.0	10.7	11.6	3.3
<i>Cocl-Lav32</i>													
<i>n</i>	18	42	46	39	30	31	55	54	34	19	29	95	40
<i>H_e</i>	0.76	0.65	0.56	0.72	0.64	0.60	0.31	0.33	0.47	0.57	0.46	0.40	0.19
<i>H_o</i>	0.72	0.71	0.52	0.69	0.53	0.52	0.25	0.31	0.44	0.68	0.45	0.39	0.20
<i>A_r</i>	6.8	6.5	6.6	8.0	6.8	6.4	4.0	2.9	5.4	7.2	6.1	5.9	3.1
<i>Cocl-Lav45</i>													
<i>N</i>	18	51	51	43	32	28	52	53	33	17	29	94	39
<i>H_e</i>	0.57	0.73	0.63	0.64	0.65	0.68	0.44	0.42	0.50	0.62	0.59	0.58	0.50
<i>H_o</i>	0.56	0.67	0.63	0.63	0.56	0.46	0.27*	0.13*	0.52	0.53	0.34	0.48	0.51
<i>A_r</i>	4.0	4.8	4.4	4.5	5.6	5.9	4.1	3.4	5.1	4.0	4.0	4.6	2.0
<i>Cocl-Lav49</i>													
<i>N</i>	21	43	50	43	32	27	55	54	34	19	26	94	40
<i>H_e</i>	0.81	0.81	0.90	0.93	0.93	0.93	0.91	0.89	0.89	0.93	0.93	0.95	0.67
<i>H_o</i>	0.81	0.74	0.90	0.93	0.97	0.96	0.91	0.87	0.97	0.95	0.88	0.97	0.68
<i>A_r</i>	9.5	9.1	14.0	16.7	13.5	15.1	12.7	13.3	12.2	13.3	13.3	15.9	6.4
<i>Cocl-Lav61</i>													
<i>N</i>	22	52	51	45	32	31	54	54	34	18	29	91	40
<i>H_e</i>	0.55	0.70	0.88	0.91	0.85	0.88	0.82	0.81	0.90	0.88	0.88	0.85	0.73
<i>H_o</i>	0.59	0.67	0.82	0.82	0.81	0.87	0.81	0.87	0.85	0.89	0.83	0.88	0.83
<i>A_r</i>	5.6	8.2	11.4	13.0	10.2	10.5	8.3	8.2	11.8	9.9	12.3	10.6	6.9

Table 3. cont.

Locus	Region/Location												
	Arc HW1	Arc HW2	Kot HW3	Kot HW4	Yuk HW5	Yuk HW6	Yuk HW7	Yuk HW8	Yuk HW9	Yuk HW10	Yuk HW11	Kus HW12	SC HW13
<i>Cocl-Lav68</i>													
<i>n</i>	23	51	52	44	32	30	55	53	34	19	30	83	38
<i>H_e</i>	0.59	0.57	0.06	0.02	0.03	0.10	0.02	0.06	0.09	0.05	0.07	0.14	0.19
<i>H_o</i>	0.61	0.53	0.06	0.02	0.03	0.03	0.02	0.06	0.09	0.05	0.07	0.13	0.21
<i>A_r</i>	3.0	4.1	1.8	1.4	1.5	2.3	1.3	1.8	2.2	1.8	2.1	2.6	2.0
<i>Sfo8a</i>													
<i>n</i>	23	52	53	44	32	29	55	54	34	19	24	97	40
<i>H_e</i>	0.79	0.68	0.76	0.72	0.68	0.72	0.75	0.70	0.77	0.76	0.69	0.75	0.68
<i>H_o</i>	0.83	0.63	0.75	0.77	0.75	0.76	0.75	0.74	0.79	0.63	0.67	0.89	0.75
<i>A_r</i>	6.4	6.7	6.1	6.7	6.2	5.9	6.2	5.5	5.0	5.8	5.3	6.1	4.8
<i>Sfo8b</i>													
<i>n</i>	23	51	52	44	32	30	55	54	34	19	25	98	39
<i>H_e</i>	0.66	0.79	0.69	0.67	0.78	0.76	0.63	0.68	0.81	0.80	0.72	0.77	0.64
<i>H_o</i>	0.52	0.80	0.63	0.61	0.78	0.77	0.60	0.63	0.85	0.74	0.56	0.73	0.56
<i>A_r</i>	6.2	6.0	5.2	5.1	5.0	6.4	4.8	5.0	6.8	6.7	4.6	6.5	4.6
avg													
<i>n</i>	22	50	51	43	32	29	54	54	33	18	28	94	39
<i>H_e</i>	0.69	0.71	0.69	0.69	0.68	0.68	0.60	0.61	0.65	0.67	0.65	0.64	0.52
<i>H_o</i>	0.68	0.68	0.70	0.67	0.67	0.63	0.59	0.59	0.67	0.67	0.59	0.66	0.52
<i>A_r</i>	6.2	6.7	7.6	7.8	7.2	7.4	6.2	6.1	6.8	6.8	6.9	7.2	4.3

^aA bold value indicates $P < 0.05$ that the sample/locus conforms to Hardy-Weinberg expectation. An asterisk (*) indicates P -value was judged significant when the α -level (0.05) was adjusted for simultaneous tests (see text).

^bAllele richness estimates are based on a minimum sample size of 16 diploid individuals.

Table 4. A matrix of F_{ST} (below diagonal) and G -test P -values (above diagonal) for humpback whitefish aggregations from five regions in Alaska. Region abbreviations are Arctic (Arc), Kotzebue Sound (Kot), Yukon River (Yuk), Kuskokwim River (Kus), southcentral Alaska (SC). Sample abbreviations are Colville River (HW1), Canning River (HW2), Selawik River Delta 2003 (HW3), Selawik River Delta 2004 (HW4), Yukon River mainstem at Rapids 1999 (HW5), Yukon River mainstem at Rapids 2004 (HW6), Fish Lake (HW7) Moose Creek (HW8), South Fork Koyukuk River (HW9), Kanuti River 2003 (HW10), Kanuti River 2004 (HW11), Whitefish Lake (HW12), Paxson Lake (HW13). Bold P -values were judged significant after adjusting α for multiple tests.

Region/Location													
	Arc HW1	Arc HW2	Kot HW3	Kot HW4	Yuk HW5	Yuk HW6	Yuk HW7	Yuk HW8	Yuk HW9	Yuk HW10	Yuk HW11	Kus HW12	SC HW13
HW1	---	<0.001											
HW2	0.057	0	<0.001										
HW3	0.145	0.083	0	0.364	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
HW4	0.158	0.099	0.005	0	<0.001								
HW5	0.160	0.109	0.022	0.025	0	0.513	<0.001	0.072	0.214	0.678	0.869	<0.001	<0.001
HW6	0.166	0.116	0.023	0.018	0.003	0	<0.001	0.004	0.040	0.984	0.785	<0.001	<0.001
HW7	0.200	0.122	0.047	0.049	0.026	0.028	0	1.000	<0.001	0.003	<0.001	<0.001	<0.001
HW8	0.191	0.120	0.043	0.043	0.020	0.024	-0.001	0	<0.001	<0.001	<0.001	<0.001	<0.001
HW9	0.160	0.100	0.029	0.032	0.007	0.011	0.016	0.007	0	0.200	0.020	<0.001	<0.001
HW10	0.176	0.118	0.027	0.025	0.001	-0.002	0.016	0.011	-0.003	0	0.162	0.003	<0.001
HW11	0.165	0.112	0.021	0.022	-0.002	-0.001	0.026	0.020	0.004	0.000	0	0.001	<0.001
HW12	0.167	0.107	0.038	0.039	0.020	0.024	0.031	0.029	0.018	0.021	0.014	0	<0.001
HW13	0.253	0.174	0.132	0.149	0.134	0.140	0.108	0.103	0.104	0.134	0.141	0.143	0

Table 5. Estimates of hierarchical gene diversity from AMOVA. Results are shown for objective 1 (all samples) and objective 2 (Yukon River samples). An asterisk (*) denotes $P < 0.001$ the value is not greater than zero; RT = between regions; SR = within regions.

Objective	Source of variation	Percent of total		
		σ^2	F_{ST}	F_{RT}
1 (5 regions / 13 pops)	Total	4.620	100.00	
	Within populations	4.248	91.951	
	Among populations	0.372	8.049	0.080*
	Among regions	0.301	6.510	0.065*
	Among populations within regions	0.071	1.539	0.016*
2 (3 regions / 7 pops)	Total	4.274	100.00	
	Within populations	4.209	98.471	
	Among populations	0.065	1.529	0.015*
	Among regions	0.061	1.418	0.014*
	Among populations within regions	0.005	0.111	0.001

Table 6. Summary of the step-wise RFLP assay for identification of eight whitefish species based on mtDNA sequence variation in the COI gene. Enzymes providing positive species identification are denoted with a Y^a. The test result shows the proportion of individuals correctly assigned to species in a blind test.

Step: enzyme	Group	Species	cut sites	COI fragments (bp)	species ID	Test result
1: <i>RsaI</i>	RsaI - 1	Pygmy whitefish	2	343-233-130	Y	6/6
	RsaI - 2	Inconnu	1	552-154	Y	6/6
	RsaI - 3	Round whitefish	0			
		Least cisco	0			
		Arctic cisco	0			
		Bering cisco	0			
		Broad whitefish	0			
		Humpback whitefish	0			
2: <i>HaeIII</i>	HaeIII - 1	Round whitefish	5	295-162-110-81-42-16	Y	6/6
	HaeIII - 2	Least cisco	5	251-152-87-81-75-60	Y	5/5 ^b
	HaeIII - 3	Arctic cisco	5	295-152-87-81-75-16		
		Bering cisco	5	295-152-87-81-75-16		
	HaeIII - 4	Broad whitefish	5	173-152-141-87-78-75		
		Humpback whitefish	5	173-152-141-87-78-75		
3a: <i>BseRI</i>	BseRI - 1	Arctic cisco	0		Y	6/6
	BseRI - 2	Bering cisco	1	378-328	Y	5/6 ^c
3b: <i>BsmI</i>	BsmI - 1	Broad whitefish	1	449-257	Y	6/6
	BsmI - 2	Humpback whitefish	0			6/6

^aHumpback and Lake whitefish are not distinguishable and are considered a single group in this test.

^bNo PCR product was observed for one individual after two attempts at amplification so the individual was dropped from the test.

^cOne putative Bering cisco was twice identified as an Arctic cisco in separate assays following independent DNA extraction.

Appendix 1. Restriction enzyme fragment lengths for the COI gene in Arctic cisco.

Enzyme	Restriction site	Fragment length (bp)	
1 cut:			
Acc36I	ACCTGC	593	113
AccBSI	CCG/CTC	617	89
AhaIII	TTT/AAA	618	88
Apal	GGGCC/C	465	241
AseI	AT/TAAT	488	218
BalI	TGG/CCA	690	16
BbvI	GCAGC	593	113
BccI	CCATC	699	7
BclI	T/GATCA	582	124
BfaI	C/TAG	578	128
BfiI	ACTGGG	514	192
BfuAI	ACCTGC	593	113
BmrI	ACTGGG	514	192
BscGI	CCCGT	415	291
BseII	ACTGG	510	196
BseNI	ACTGG	510	196
BseXI	GCAGC	593	113
Bsp120I	G/GGCC	469	237
BspMI	ACCTGC	593	113
BsrBI	CCG/CTC	617	89
BsrI	ACTGG	510	196
BsrSI	ACTGG	510	196
BstV1I	GCAGC	593	113
BveI	ACCTGC	593	113
Cfr9I	C/CCGGG	602	104
DraI	TTT/AAA	618	88
EsaBC3I	TC/GA	403	303
FbaI	T/GATCA	582	124
FspBI	C/TAG	578	128
Ksp22I	T/GATCA	582	124
MaeI	C/TAG	578	128
MbiI	CCG/CTC	617	89
MlsI	TGG/CCA	690	16
MluNI	TGG/CCA	690	16
MroNI	G/CCGGC	368	338
MscI	TGG/CCA	690	16
Msp20I	TGG/CCA	690	16
Nael	GCC/GGC	366	340
NgoMIV	G/CCGGC	368	338
PdiI	GCC/GGC	366	340
PshBI	AT/TAAT	488	218
PspAI	C/CCGGG	602	104
PspOMI	G/GGCC	469	237
SapI	GCTCTTC	589	117

Appendix 1. Restriction enzyme fragment lengths for the COI gene in Arctic cisco.

Enzyme	Restriction site	Fragment length (bp)			
SimI	GG/GTC	636	70		
SmaI	CCC/GGG	600	106		
SspI	AAT/ATT	677	29		
TaqI	T/CGA	402	304		
TspGWI	ACGGA	413	293		
UthSI	CC/CGGG	601	105		
VspI	AT/TAAT	488	218		
XmaCI	C/CCGGG	602	104		
XmaI	C/CCGGG	602	104		
XspI	C/TAG	578	128		
2 cuts:					
AccIII	T/CCGGA	374	320	12	
AclWI	GGATC	661	40	5	
AlwI	GGATC	661	40	5	
Aor13HI	T/CCGGA	374	320	12	
BinI	GGATC	661	40	5	
BlfI	T/CCGGA	374	320	12	
BseAI	T/CCGGA	374	320	12	
BseMII	CTCAG	483	163	60	
BseYI	C/CCAGC	493	144	69	
Bsp13I	T/CCGGA	374	320	12	
BspCNI	CTCAG	483	164	59	
BspEI	T/CCGGA	374	320	12	
BspMII	T/CCGGA	374	320	12	
BspNCI	CCAGA	340	252	114	
BspPI	GGATC	661	40	5	
Bst6I	CTCTTC	563	117	26	
CstMI	AAGGAG	379	309	18	
Eam1104I	CTCTTC	563	117	26	
EarI	CTCTTC	563	117	26	
FauI	CCCGC	649	53	4	
FokI	GGATG	346	226	134	
Kpn2I	T/CCGGA	374	320	12	
Ksp632I	CTCTTC	563	117	26	
MboII	GAAGA	563	124	19	
MroI	T/CCGGA	374	320	12	
PsiI	TTA/TAA	476	135	95	
SmuI	CCCGC	649	53	4	
StsI	GGATG	345	226	135	
3 cuts:					
AluI	AG/CT	276	209	155	66
BceAI	ACGGC	306	165	157	78
Bcefl	ACGGC	306	165	157	78
BfuCI	GATC	528	123	50	5
BseGI	GGATG	345	226	127	8
Bsp143I	GATC	528	123	50	5

Appendix 1. Restriction enzyme fragment lengths for the COI gene in Arctic cisco.

Enzyme	Restriction site	Fragment length (bp)							
BstF5I	GGATG	345	226	127	8				
BstKTI	GAT/C	528	127	46	5				
BstMBI	GATC	528	123	50	5				
ChaI	GATC	528	128	45	5				
CviAII	C/ATG	247	237	111	111				
DpnI	GA/TC	528	126	47	5				
DpnII	GATC	528	123	50	5				
FatI	CATG	245	237	113	111				
Hin1II	CATG	250	237	111	108				
Hsp92II	CATG	250	237	111	108				
Kzo9I	GATC	528	123	50	5				
Mall	GA/TC	528	126	47	5				
MboI	GATC	528	123	50	5				
NdeII	GATC	528	123	50	5				
NlaIII	CATG	250	237	111	108				
Sau3AI	GATC	528	123	50	5				
TspDTI	ATGAA	278	206	136	86				
4 cuts:									
AciI	C/CGC	457	95	89	61	4			
SsiI	C/CGC	457	95	89	61	4			
5 cuts:									
BshFI	GG/CC	295	152	87	81	75			
BspANI	GG/CC	295	152	87	81	75			
BsuRI	GG/CC	295	152	87	81	75			
HaeIII	GG/CC	295	152	87	81	75			
Hin4II	CCTTC	249	158	139	127	18			
HpyAV	CCTTC	249	169	128	127	18			
PalI	GG/CC	295	152	87	81	75			
PhoI	GG/CC	295	152	87	81	75			
Sse9I	AATT	241	188	94	80	73			
TasI	AATT	241	188	94	80	73			
Tsp509I	AATT	241	188	94	80	73			
TspEI	AATT	241	188	94	80	73			
6 cuts:									
7 cuts:									
BsiSI	C/CGG	271	216	85	54	51	12	11	6
HapII	C/CGG	271	216	85	54	51	12	11	6
HpaII	C/CGG	271	216	85	54	51	12	11	6
MseI	T/TAA	189	138	89	80	78	59	52	21
MspI	C/CGG	271	216	85	54	51	12	11	6
Sth302II	CC/GG	271	216	85	54	52	12	10	6
Tru1I	T/TAA	189	138	89	80	78	59	52	21
Tru9I	T/TAA	189	138	89	80	78	59	52	21

Appendix 1. Restriction enzyme fragment lengths for the COI gene in Arctic cisco.

Enzyme	Restriction site	Fragment length (bp)								
8 cuts:										
MnlII	CCTC	211	178	114	77	50	25	20	17	14
Sth132I	CCCG	350	129	111	53	34	16	7	4	2

Appendix 2. Restriction enzyme fragment lengths for the COI gene in Bering cisco.

Enzyme	Restriction site	Fragment length (bp)								
1 cut:										
Acc36I	ACCTGC	593	113							
AccBSI	CCG/CTC	617	89							
AhaIII	TTT/AAA	618	88							
Apal	GGGCC/C	465	241							
AseI	AT/TAAT	488	218							
BalII	TGG/CCA	690	16							
BbvI	GCAGC	593	113							
BccI	CCATC	699	7							
BclII	T/GATCA	582	124							
BfaI	C/TAG	578	128							
BfiI	ACTGGG	514	192							
BfuAI	ACCTGC	593	113							
BmrI	ACTGGG	514	192							
BscGI	CCCGT	415	291							
BseII	ACTGG	510	196							
BseNI	ACTGG	510	196							
BseRI	GAGGAG	378	328							
BseXI	GCAGC	593	113							
Bsp120I	G/GGCC	469	237							
BspMI	ACCTGC	593	113							
BsrBI	CCG/CTC	617	89							
BsrI	ACTGG	510	196							
BsrSI	ACTGG	510	196							
BstVII	GCAGC	593	113							
BveI	ACCTGC	593	113							
Cfr9I	C/CCGGG	602	104							
DraI	TTT/AAA	618	88							
EsaBC3I	TC/GA	403	303							
FbaI	T/GATCA	582	124							
FspBI	C/TAG	578	128							
Ksp22I	T/GATCA	582	124							
MaeI	C/TAG	578	128							
MbiI	CCG/CTC	617	89							
MlsI	TGG/CCA	690	16							
MluNI	TGG/CCA	690	16							
MroNI	G/CCGGC	368	338							
MscI	TGG/CCA	690	16							

Appendix 2. Restriction enzyme fragment lengths for the COI gene in Bering cisco.

Enzyme	Restriction site	Fragment length (bp)	
Msp20I	TGG/CCA	690	16
NaeI	GCC/GGC	366	340
NgoMIV	G/CCGGC	368	338
PdiI	GCC/GGC	366	340
PshBI	AT/TAAT	488	218
PspAI	C/CCGGG	602	104
PspOMI	G/GGCC	469	237
SapI	GCTCTTC	589	117
SimI	GG/GTC	636	70
SmaI	CCC/GGG	600	106
SspI	AAT/ATT	677	29
TaqI	T/CGA	402	304
TspGWI	ACGGA	413	293
UthSI	CC/CGGG	601	105
VspI	AT/TAAT	488	218
XmaCI	C/CCGGG	602	104
XmaI	C/CCGGG	602	104
XspI	C/TAG	578	128
2 cuts:			
AccIII	T/CCGGA	374	320
AclWI	GGATC	661	40
AlwI	GGATC	661	40
Aor13HI	T/CCGGA	374	320
BinI	GGATC	661	40
BlfI	T/CCGGA	374	320
BseAI	T/CCGGA	374	320
BseMII	CTCAG	483	163
BseYI	C/CCAGC	493	144
Bsp13I	T/CCGGA	374	320
BspCNI	CTCAG	483	164
BspEI	T/CCGGA	374	320
BspMII	T/CCGGA	374	320
BspNCI	CCAGA	340	252
BspPI	GGATC	661	40
Bst6I	CTCTTC	563	117
CstMI	AAGGAG	379	309
Eam1104I	CTCTTC	563	117
EarI	CTCTTC	563	117
FauI	CCCGC	649	53
FokI	GGATG	346	226
Kpn2I	T/CCGGA	374	320
Ksp632I	CTCTTC	563	117
MboII	GAAGA	563	124
MroI	T/CCGGA	374	320
PsiI	TTA/TAA	476	135
SmuI	CCCGC	649	53
StsI	GGATG	345	226
			135

Appendix 2. Restriction enzyme fragment lengths for the COI gene in Bering cisco.

Enzyme	Restriction site	Fragment length (bp)				
3 cuts:						
AluI	AG/CT	276	209	155	66	
BceAI	ACGGC	306	165	157	78	
Bcefl	ACGGC	306	165	157	78	
BfuCI	GATC	528	123	50	5	
BseGI	GGATG	345	226	127	8	
Bsp143I	GATC	528	123	50	5	
BstF5I	GGATG	345	226	127	8	
BstKTI	GAT/C	528	127	46	5	
BstMBI	GATC	528	123	50	5	
Chal	GATC	528	128	45	5	
CviAII	C/ATG	247	237	111	111	
DpnI	GA/TC	528	126	47	5	
DpnII	GATC	528	123	50	5	
FatI	CATG	245	237	113	111	
Hin1II	CATG	250	237	111	108	
Hsp92II	CATG	250	237	111	108	
Kzo9I	GATC	528	123	50	5	
Mall	GA/TC	528	126	47	5	
MboI	GATC	528	123	50	5	
NdeII	GATC	528	123	50	5	
NlaIII	CATG	250	237	111	108	
Sau3AI	GATC	528	123	50	5	
TspDTI	ATGAA	278	206	136	86	
4 cuts:						
AciI	C/CGC	457	95	89	61	4
SsiI	C/CGC	457	95	89	61	4
5 cuts:						
BshFI	GG/CC	295	152	87	81	75
BspANI	GG/CC	295	152	87	81	75
BsuRI	GG/CC	295	152	87	81	75
HaeIII	GG/CC	295	152	87	81	75
Hin4II	CCTTC	249	158	139	127	18
HpyAV	CCTTC	249	169	128	127	18
PalI	GG/CC	295	152	87	81	75
PhoI	GG/CC	295	152	87	81	75
Sse9I	AATT	241	188	94	80	73
TasI	AATT	241	188	94	80	73
Tsp509I	AATT	241	188	94	80	73
TspEI	AATT	241	188	94	80	73
6 cuts:						
7 cuts:						
BsiSI	C/CGG	271	216	85	54	51
					12	11
					6	

Appendix 2. Restriction enzyme fragment lengths for the COI gene in Bering cisco.

Enzyme	Restriction site	Fragment length (bp)							
		271	216	85	54	51	12	11	6
HpaII	C/CGG	271	216	85	54	51	12	11	6
HpaII	C/CGG	271	216	85	54	51	12	11	6
MseI	T/TAA	189	138	89	80	78	59	52	21
MspI	C/CGG	271	216	85	54	51	12	11	6
Sth302II	CC/GG	271	216	85	54	52	12	10	6
Tru1I	T/TAA	189	138	89	80	78	59	52	21
Tru9I	T/TAA	189	138	89	80	78	59	52	21
8 cuts:									
Sth132I	CCCG	350	129	111	53	34	16	7	4
9 cuts:									
MnlI	CCTC	211	178	114	77	47	25	20	17
									3

Appendix 3. Restriction enzyme fragment lengths for the COI gene in broad whitefish.

Enzyme	Restriction site	Fragment length (bp)							
		593	113	617	89	661	45	661	45
1 cut:									
Acc36I	ACCTGC	593	113						
AccBSI	CCG/CTC	617	89						
AclWI	GGATC	661	45						
AlwI	GGATC	661	45						
Apal	GGGCC/C	465	241						
AseI	AT/TAAT	488	218						
BbvI	GCAGC	593	113						
BclI	T/GATCA	582	124						
BfaI	C/TAG	578	128						
BfiI	ACTGGG	514	192						
BfuAI	ACCTGC	593	113						
BinI	GGATC	661	45						
BmrI	ACTGGG	514	192						
BsaMI	GAATGC	449	257						
BsbI	CAACAC	667	39						
Bse1I	ACTGG	510	196						
BseMII	CTCAG	646	60						
BseNI	ACTGG	510	196						
BseXI	GCAGC	593	113						
BsmI	GAATGC	449	257						
Bsp120I	G/GGCC	469	237						
BspCNI	CTCAG	647	59						
BspMI	ACCTGC	593	113						
BspPI	GGATC	661	45						
BsrBI	CCG/CTC	617	89						
BsrI	ACTGG	510	196						
BsrSI	ACTGG	510	196						

Appendix 3. Restriction enzyme fragment lengths for the COI gene in broad whitefish.

Enzyme	Restriction site	Fragment length (bp)		
BstV1I	GCAGC	593	113	
BveI	ACCTGC	593	113	
Cfr9I	C/CCGGG	602	104	
EsaBC3I	TC/GA	403	303	
FbaI	T/GATCA	582	124	
FspBI	C/TAG	578	128	
Ksp22I	T/GATCA	582	124	
MaeI	C/TAG	578	128	
MbiI	CCG/CTC	617	89	
MroNI	G/CCGGC	368	338	
Mva1269I	GAATGC	449	257	
NaeI	GCC/GGC	366	340	
NgoMIV	G/CCGGC	368	338	
PctI	GAATGC	449	257	
PdiI	GCC/GGC	366	340	
PshBI	AT/TAAT	488	218	
PspAI	C/CCGGG	602	104	
PspOMI	G/GGCC	469	237	
SapI	GCTCTTC	589	117	
SimI	GG/GTC	636	70	
SmaI	CCC/GGG	600	106	
SspI	AAT/ATT	677	29	
TaqI	T/CGA	402	304	
TspGWI	ACGGA	413	293	
UthSI	CC/CGGG	601	105	
VspI	AT/TAAT	488	218	
XmaCI	C/CCGGG	602	104	
XmaI	C/CCGGG	602	104	
XspI	C/TAG	578	128	
2 cuts:				
BfuCI	GATC	528	123	55
BseYI	C/CCAGC	493	144	69
Bsp143I	GATC	528	123	55
Bst6I	CTCTTC	563	117	26
BstKTI	GAT/C	528	127	51
BstMBI	GATC	528	123	55
Chal	GATC	528	128	50
DpnI	GA/TC	528	126	52
DpnII	GATC	528	123	55
Eam1104I	CTCTTC	563	117	26
EarI	CTCTTC	563	117	26
FauI	CCCGC	649	53	4
FokI	GGATG	346	226	134
Ksp632I	CTCTTC	563	117	26
Kzo9I	GATC	528	123	55
Mall	GA/TC	528	126	52
MboI	GATC	528	123	55

Appendix 3. Restriction enzyme fragment lengths for the COI gene in broad whitefish.

Enzyme	Restriction site				Fragment length (bp)		
MboII	GAAGA	563	124	19			
NdeII	GATC	528	123	55			
PsiI	TTA/TAA	476	135	95			
Sau3AI	GATC	528	123	55			
SmuI	CCCGC	649	53	4			
StsI	GGATG	345	226	135			
3 cuts:							
AluI	AG/CT	276	209	155	66		
BceAI	ACGGC	306	165	157	78		
Bcefl	ACGGC	306	165	157	78		
BseGI	GGATG	345	226	127	8		
BstF5I	GGATG	345	226	127	8		
CstMI	AAGGAG	379	309	11	7		
CviAII	C/ATG	247	237	111	111		
FatI	CATG	245	237	113	111		
Hin1II	CATG	250	237	111	108		
Hsp92II	CATG	250	237	111	108		
NlaIII	CATG	250	237	111	108		
Sse9I	AATT	271	188	167	80		
TasI	AATT	271	188	167	80		
Tsp509I	AATT	271	188	167	80		
TspDTI	ATGAA	278	206	136	86		
TspEI	AATT	271	188	167	80		
4 cuts:							
AciI	C/CGC	457	95	89	61	4	
BspNCI	CCAGA	252	167	161	114	12	
SsiI	C/CGC	457	95	89	61	4	
5 cuts:							
BshFI	GG/CC	173	152	141	87	78	75
BspANI	GG/CC	173	152	141	87	78	75
BsuRI	GG/CC	173	152	141	87	78	75
HaeIII	GG/CC	173	152	141	87	78	75
Hin4II	CCTTC	249	158	139	127	18	15
HpyAV	CCTTC	249	169	128	127	18	15
PalI	GG/CC	173	152	141	87	78	75
PhoI	GG/CC	173	152	141	87	78	75
6 cuts:							
BsiSI	C/CGG	271	216	96	54	51	12
HpaII	C/CGG	271	216	96	54	51	12
HpaII	C/CGG	271	216	96	54	51	12
MseI	T/TAA	189	167	138	80	59	52
MspI	C/CGG	271	216	96	54	51	12
Sth302II	CC/GG	271	216	95	54	52	12
TruII	T/TAA	189	167	138	80	59	52
							21

Appendix 3. Restriction enzyme fragment lengths for the COI gene in broad whitefish.

Enzyme	Restriction site	Fragment length (bp)										
		189	167	138	80	59	52	21				
Tru9I	T/TAA											
7 cuts:												
8 cuts:												
Sth132I	CCCG	320	129	111	64	53	16	7	4	2		
9 cuts:												
10 cuts:												
MnlII	CCTC	211	155	93	77	50	25	23	21	20	17	14

Appendix 4. Restriction enzyme fragment lengths for the COI gene in humpback whitefish.

Enzyme	Restriction site	Fragment length (bp)	
		1	2
1 cut:			
Acc36I	ACCTGC	593	113
AccBSI	CCG/CTC	617	89
AclWI	GGATC	661	45
AlwI	GGATC	661	45
ApaI	GGGCC/C	465	241
AseI	AT/TAAT	488	218
BbvI	GCAGC	593	113
BclII	T/GATCA	582	124
BfaI	C/TAG	578	128
BfiI	ACTGGG	514	192
BfuAI	ACCTGC	593	113
BinI	GGATC	661	45
BmrI	ACTGGG	514	192
BsbI	CAACAC	667	39
Bse1I	ACTGG	510	196
BseMII	CTCAG	646	60
BseNI	ACTGG	510	196
BseXI	GCAGC	593	113
Bsp120I	G/GGCC	469	237
BspCNI	CTCAG	647	59
BspMI	ACCTGC	593	113
BspPI	GGATC	661	45
BsrBI	CCG/CTC	617	89
BsrI	ACTGG	510	196
BsrSI	ACTGG	510	196
BstV1I	GCAGC	593	113
BtsI	GCAGTG	555	151
BveI	ACCTGC	593	113
Cfr9I	C/CCGGG	602	104

Appendix 4. Restriction enzyme fragment lengths for the COI gene in humpback whitefish.

Enzyme	Restriction site	Fragment length (bp)		
EsaBC3I	TC/GA	403	303	
FbaI	T/GATCA	582	124	
FspBI	C/TAG	578	128	
Ksp22I	T/GATCA	582	124	
MaeI	C/TAG	578	128	
MbiI	CCG/CTC	617	89	
MroNI	G/CCGGC	368	338	
NaeI	GCC/GGC	366	340	
NgoMIV	G/CCGGC	368	338	
PdiI	GCC/GGC	366	340	
PshBI	AT/TAAT	488	218	
PspAI	C/CCGGG	602	104	
PspOMI	G/GGCC	469	237	
SapI	GCTCTTC	589	117	
SimI	GG/GTC	636	70	
SmaI	CCC/GGG	600	106	
SspI	AAT/ATT	677	29	
TaqI	T/CGA	402	304	
TspGWI	ACGGA	413	293	
UthSI	CC/CGGG	601	105	
VspI	AT/TAAT	488	218	
XmaCI	C/CCGGG	602	104	
XmaI	C/CCGGG	602	104	
XspI	C/TAG	578	128	
2 cuts:				
AccIII	T/CCGGA	374	320	12
Aor13HI	T/CCGGA	374	320	12
BfuCI	GATC	528	123	55
BlfI	T/CCGGA	374	320	12
BseAI	T/CCGGA	374	320	12
BseYI	C/CCAGC	493	144	69
Bsp13I	T/CCGGA	374	320	12
Bsp143I	GATC	528	123	55
BspEI	T/CCGGA	374	320	12
BspMII	T/CCGGA	374	320	12
Bst6I	CTCTTC	563	117	26
BstKTI	GAT/C	528	127	51
BstMBI	GATC	528	123	55
ChaI	GATC	528	128	50
DpnI	GA/TC	528	126	52
DpnII	GATC	528	123	55
Eam1104I	CTCTTC	563	117	26
EarI	CTCTTC	563	117	26
FauI	CCCGC	649	53	4
FokI	GGATG	346	226	134
Kpn2I	T/CCGGA	374	320	12
Ksp632I	CTCTTC	563	117	26

Appendix 4. Restriction enzyme fragment lengths for the COI gene in humpback whitefish.

Enzyme	Restriction site	Fragment length (bp)						
Kzo9I	GATC	528	123	55				
Mall	GA/TC	528	126	52				
MboI	GATC	528	123	55				
MboII	GAAGA	563	124	19				
MroI	T/CCGGA	374	320	12				
NdeII	GATC	528	123	55				
PsiI	TTA/TAA	476	135	95				
Sau3AI	GATC	528	123	55				
SmuI	CCCGC	649	53	4				
StsI	GGATG	345	226	135				
3 cuts:								
AluI	AG/CT	276	209	155	66			
BceAI	ACGGC	306	165	157	78			
BceFI	ACGGC	306	165	157	78			
BseGI	GGATG	345	226	127	8			
BspNCI	CCAGA	252	179	161	114			
BstF5I	GGATG	345	226	127	8			
CstMI	AAGGAG	379	309	11	7			
CviAII	C/ATG	247	237	111	111			
FatI	CATG	245	237	113	111			
Hin1II	CATG	250	237	111	108			
Hsp92II	CATG	250	237	111	108			
NlaIII	CATG	250	237	111	108			
Sse9I	AATT	271	188	167	80			
TasI	AATT	271	188	167	80			
Tsp509I	AATT	271	188	167	80			
TspDTI	ATGAA	278	206	136	86			
TspEI	AATT	271	188	167	80			
4 cuts:								
AciI	C/CGC	457	95	89	61	4		
Hin4II	CCTTC	264	158	139	127	18		
HpyAV	CCTTC	264	169	128	127	18		
SsiI	C/CGC	457	95	89	61	4		
5 cuts:								
BshFI	GG/CC	173	152	141	87	78	75	
BspANI	GG/CC	173	152	141	87	78	75	
BsuRI	GG/CC	173	152	141	87	78	75	
HaeIII	GG/CC	173	152	141	87	78	75	
PalI	GG/CC	173	152	141	87	78	75	
PhoI	GG/CC	173	152	141	87	78	75	
6 cuts:								
MseI	T/TAA	189	167	138	80	59	52	21
Tru1I	T/TAA	189	167	138	80	59	52	21
Tru9I	T/TAA	189	167	138	80	59	52	21

Appendix 4. Restriction enzyme fragment lengths for the COI gene in humpback whitefish.

Enzyme	Restriction site	Fragment length (bp)										
7 cuts:												
BsiSI	C/CGG	271	216	85	54	51	12	11	6			
HapII	C/CGG	271	216	85	54	51	12	11	6			
HpaII	C/CGG	271	216	85	54	51	12	11	6			
MspI	C/CGG	271	216	85	54	51	12	11	6			
Sth132I	CCCG	384	129	111	53	16	7	4	2			
Sth302II	CC/GG	271	216	85	54	52	12	10	6			
8 cuts:												
9 cuts:												
10 cuts:												
MnII	CCTC	211	155	93	77	50	25	23	21	20	17	14

Appendix 5. Restriction enzyme fragment lengths for the COI gene in least cisco.

Enzyme	Restriction site	Fragment length (bp)								
1 cut:										
Acc36I	ACCTGC	593	113							
AccBSI	CCG/CTC	617	89							
AclWI	GGATC	661	45							
AlwI	GGATC	661	45							
ApaI	GGGCC/C	465	241							
AseI	AT/TAAT	488	218							
BbvI	GCAGC	593	113							
BclI	T/GATCA	582	124							
BfaI	C/TAG	578	128							
BfiI	ACTGGG	514	192							
BfuAI	ACCTGC	593	113							
BinI	GGATC	661	45							
BmrI	ACTGGG	514	192							
BseII	ACTGG	510	196							
BseNI	ACTGG	510	196							
BseRI	GAGGAG	663	43							
BseXI	GCAGC	593	113							
Bsp120I	G/GGCC	469	237							
BspMI	ACCTGC	593	113							
BspPI	GGATC	661	45							
BsrBI	CCG/CTC	617	89							
BsrI	ACTGG	510	196							
BsrSI	ACTGG	510	196							
BstV1I	GCAGC	593	113							
BveI	ACCTGC	593	113							

Appendix 5. Restriction enzyme fragment lengths for the COI gene in least cisco.

Enzyme	Restriction site	Fragment length (bp)	
Cfr9I	C/CCGGG	602	104
EciI	GGCGGA	660	46
FauI	CCCGC	649	57
FbaI	T/GATCA	582	124
FspBI	C/TAG	578	128
Ksp22I	T/GATCA	582	124
MaeI	C/TAG	578	128
MbiI	CCG/CTC	617	89
MroNI	G/CCGGC	368	338
NaeI	GCC/GGC	366	340
NgoMIV	G/CCGGC	368	338
PdiI	GCC/GGC	366	340
PshBI	AT/TAAT	488	218
PspAI	C/CCGGG	602	104
PspOMI	G/GGCC	469	237
SapI	GCTCTTC	589	117
SimI	GG/GTC	636	70
SmaI	CCC/GGG	600	106
SmuI	CCCGC	649	57
SspI	AAT/ATT	677	29
TspGWI	ACGGA	413	293
UthSI	CC/CGGG	601	105
VspI	AT/TAAT	488	218
XmaCI	C/CCGGG	602	104
XmaI	C/CCGGG	602	104
XspI	C/TAG	578	128
2 cuts:			
AccIII	T/CCGGA	374	320 12
AluI	AG/CT	276	275 155
Aor13HI	T/CCGGA	374	320 12
BfuCI	GATC	528	123 55
BlfI	T/CCGGA	374	320 12
BseAI	T/CCGGA	374	320 12
BseMII	CTCAG	483	163 60
BseYI	C/CCAGC	493	144 69
Bsp13I	T/CCGGA	374	320 12
Bsp143I	GATC	528	123 55
BspCNI	CTCAG	483	164 59
BspEI	T/CCGGA	374	320 12
BspMII	T/CCGGA	374	320 12
BspNCI	CCAGA	340	252 114
Bst6I	CTCTTC	563	117 26
BstKTI	GAT/C	528	127 51
BstMBI	GATC	528	123 55
ChaI	GATC	528	128 50
DpnI	GA/TC	528	126 52
DpnII	GATC	528	123 55

Appendix 5. Restriction enzyme fragment lengths for the COI gene in least cisco.

Enzyme	Restriction site	Fragment length (bp)			
Eam1104I	CTCTTC	563	117	26	
EarI	CTCTTC	563	117	26	
EsaBC3I	TC/GA	403	231	72	
FokI	GGATG	346	226	134	
Kpn2I	T/CCGGA	374	320	12	
Ksp632I	CTCTTC	563	117	26	
Kzo9I	GATC	528	123	55	
Mall	GA/TC	528	126	52	
MboI	GATC	528	123	55	
MroI	T/CCGGA	374	320	12	
NdeII	GATC	528	123	55	
PsiI	TTA/TAA	476	135	95	
Sau3AI	GATC	528	123	55	
StsI	GGATG	345	226	135	
TaqI	T/CGA	402	231	73	
3 cuts:					
BceAI	ACGGC	306	165	157	78
BceFI	ACGGC	306	165	157	78
BseGI	GGATG	345	226	127	8
BstF5I	GGATG	345	226	127	8
CstMI	AAGGAG	379	309	11	7
CviAII	C/ATG	247	237	111	111
FatI	CATG	245	237	113	111
Hin1II	CATG	250	237	111	108
Hsp92II	CATG	250	237	111	108
MboII	GAAGA	518	124	45	19
NlaIII	CATG	250	237	111	108
Sse9I	AATT	271	188	167	80
TasI	AATT	271	188	167	80
Tsp509I	AATT	271	188	167	80
TspDTI	ATGAA	278	206	136	86
TspEI	AATT	271	188	167	80
4 cuts:					
AciI	C/CGC	457	95	89	61
SsiI	C/CGC	457	95	89	61
5 cuts:					
BshFI	GG/CC	251	152	87	81
BspANI	GG/CC	251	152	87	81
BsuRI	GG/CC	251	152	87	81
HaeIII	GG/CC	251	152	87	81
Hin4II	CCTTC	249	158	139	127
HpyAV	CCTTC	249	169	128	127
PaiI	GG/CC	251	152	87	81
PhoI	GG/CC	251	152	87	81

Appendix 5. Restriction enzyme fragment lengths for the COI gene in least cisco.

Enzyme	Restriction site	Fragment length (bp)							
6 cuts:									
MseI	T/TAA	189	167	138	80	59	52	21	
Sth132I	CCCG	384	129	111	57	16	7	2	
Tru1I	T/TAA	189	167	138	80	59	52	21	
Tru9I	T/TAA	189	167	138	80	59	52	21	
7 cuts:									
BsiSI	C/CGG	271	216	85	54	51	12	11	6
HapII	C/CGG	271	216	85	54	51	12	11	6
HpaII	C/CGG	271	216	85	54	51	12	11	6
MspI	C/CGG	271	216	85	54	51	12	11	6
Sth302II	CC/GG	271	216	85	54	52	12	10	6
8 cuts:									
9 cuts:									
10 cuts:									
11 cuts:									
MnlII	CCTC	211	130	93	77	50	25	25	23
							21	20	17

Appendix 6. Restriction enzyme fragment lengths for the COI gene in pygmy whitefish.

Enzyme	Restriction site	Fragment length (bp)							
1 cut:									
AccBSI	CCG/CTC	617	89						
AcI III	CAGCTC	543	163						
Alw26I	GTCTC	656	50						
ApaI	GGGCC/C	594	112						
AseI	AT/TAAT	488	218						
AspLEI	GCG/C	368	338						
AsuHPI	GGTGA	703	3						
BbeI	GGCGC/C	367	339						
BbvCI	CC/TCAGC	635	71						
BbvI	GCAGC	593	113						
BccI	CCATC	506	200						
BclI	T/GATCA	582	124						
BsaI	GGTCTC	657	49						
BseII	ACTGG	582	124						
BseGI	GGATG	353	353						
BseNI	ACTGG	582	124						
BseXI	GCAGC	593	113						
BsmAI	GTCTC	656	50						
Bso31I	GGTCTC	657	49						

Appendix 6. Restriction enzyme fragment lengths for the COI gene in pygmy whitefish.

Enzyme	Restriction site	Fragment length (bp)	
Bsp120I	G/GGCC	598	108
BspTNI	GGTCTC	657	49
BsrBI	CCG/CTC	617	89
BsrI	ACTGG	582	124
BsrSI	ACTGG	582	124
BstF5I	GGATG	353	353
BstHHI	GCG/C	368	338
BstMAI	GTCTC	656	50
BstV1I	GCAGC	593	113
CfoI	GCG/C	368	338
Cfr9I	C/CCGGG	602	104
CviAII	C/ATG	522	184
DrdII	GAACCA	679	27
Eco31II	GGTCTC	657	49
EgeI	GGC/GCC	369	337
EheI	GGC/GCC	369	337
FatI	CATG	524	182
FbaI	T/GATCA	582	124
FokI	GGATG	360	346
HhaI	GCG/C	368	338
Hin1II	CATG	519	187
Hin6I	G/CGC	370	336
HinP1I	G/CGC	370	336
HindIII	A/AGCTT	432	274
HphI	GGTGA	703	3
Hsp92II	CATG	519	187
HspAI	G/CGC	370	336
KasI	G/GGCC	371	335
Ksp22I	T/GATCA	582	124
MbiI	CCG/CTC	617	89
Mly113I	GG/CGCC	370	336
MroNI	G/CCGGC	374	332
NaeI	GCC/GGC	372	334
NarI	GG/CGCC	370	336
NgoMIV	G/CCGGC	374	332
NlaIII	CATG	519	187
PdiI	GCC/GGC	372	334
PshBI	AT/TAAT	488	218
PsiI	TTA/TAA	571	135
PspAI	C/CCGGG	602	104
PspOMI	G/GGCC	598	108
SfoI	GGC/GCC	369	337
SmaI	CCC/GGG	600	106
SspD5I	GGTGA	703	3
StsI	GGATG	361	345
TspGWI	ACGGA	413	293
UthSI	CC/CCGGG	601	105
VspI	AT/TAAT	488	218

Appendix 6. Restriction enzyme fragment lengths for the COI gene in pygmy whitefish.

Enzyme	Restriction site	Fragment length (bp)		
XmaCI	C/CCGGG	602	104	
XmaI	C/CCGGG	602	104	
2 cuts:				
AfaI	GT/AC	343	233	130
BceAI	ACGGC	308	235	163
Bcefl	ACGGC	308	235	163
BscGI	CCCGT	314	257	135
BseMII	CTCAG	621	60	25
BseYI	C/CCAGC	493	144	69
BshFI	GG/CC	392	204	110
BspANI	GG/CC	392	204	110
BspCNI	CTCAG	622	59	25
BspNCI	CCAGA	375	319	12
BsuRI	GG/CC	392	204	110
Csp6I	G/TAC	342	233	131
EciI	GGCGGA	329	305	72
HaeIII	GG/CC	392	204	110
Hin4II	CCTTC	409	158	139
HpyAV	CCTTC	409	169	128
PalI	GG/CC	392	204	110
PhoI	GG/CC	392	204	110
RsaI	GT/AC	343	233	130
SimI	GG/GTC	636	52	18
TspDTI	ATGAA	364	206	136
3 cuts:				
AluI	AG/CT	276	173	150
BfaI	C/TAG	336	326	32
BfuCI	GATC	466	123	100
Bsp143I	GATC	466	123	100
BstKTI	GAT/C	466	127	96
BstMBI	GATC	466	123	100
ChaI	GATC	466	128	95
DpnI	GA/TC	466	126	97
DpnII	GATC	466	123	100
FauI	CCCGC	379	153	114
FspBI	C/TAG	336	326	32
Kzo9I	GATC	466	123	100
MaeI	C/TAG	336	326	32
MaiI	GA/TC	466	126	97
MboI	GATC	466	123	100
MboII	GAAGA	390	298	15
NdeII	GATC	466	123	100
Sau3AI	GATC	466	123	100
SmuI	CCCGC	379	153	114
XspI	C/TAG	336	326	32

Appendix 6. Restriction enzyme fragment lengths for the COI gene in pygmy whitefish.

Enzyme	Restriction site	Fragment length (bp)									
4 cuts:											
BsiSI	C/CGG	367	228	54	51	6					
HapII	C/CGG	367	228	54	51	6					
HpaII	C/CGG	367	228	54	51	6					
MseI	T/TAA	247	189	138	80	52					
MspI	C/CGG	367	228	54	51	6					
Sse9I	AATT	271	188	117	80	50					
Sth302II	CC/GG	366	228	54	52	6					
TasI	AATT	271	188	117	80	50					
Tru1I	T/TAA	247	189	138	80	52					
Tru9I	T/TAA	247	189	138	80	52					
Tsp509I	AATT	271	188	117	80	50					
TspEI	AATT	271	188	117	80	50					
5 cuts:											
6 cuts:											
7 cuts:											
AciI	C/CGC	201	122	115	89	72	60	38	9		
SsiI	C/CGC	201	122	115	89	72	60	38	9		
8 cuts:											
Sth132I	CCCG	140	136	114	114	111	60	16	13	2	
9 cuts:											
MnlI	CCTC	224	114	89	80	50	42	31	28	25	23

Appendix 7. Restriction enzyme fragment lengths for the COI gene in round whitefish.

Enzyme	Restriction site	Fragment length (bp)					
1 cut:							
Acc36I	ACCTGC	593	113				
AccBSI	CCG/CTC	617	89				
AccIII	T/CCGGA	386	320				
AclWI	GGATC	616	90				
Alw26I	GTCTC	656	50				
AlwI	GGATC	616	90				
Aor13HI	T/CCGGA	386	320				
Apal	GGGCC/C	594	112				
AseI	AT/TAAT	480	226				
AspLEI	GCG/C	368	338				
AsuHPI	GGTGA	555	151				
BalI	TGG/CCA	690	16				
BbeI	GGCGC/C	367	339				

Appendix 7. Restriction enzyme fragment lengths for the COI gene in round whitefish.

Enzyme	Restriction site		Fragment length (bp)
BbvI	GCAGC	593	113
BciVI	GTATCC	369	337
BfaI	C/TAG	368	338
BfuAI	ACCTGC	593	113
BfuI	GTATCC	369	337
BinI	GGATC	616	90
BlfI	T/CCGGA	386	320
BsaI	GGTCTC	657	49
BsaMI	GAATGC	449	257
BsbI	CAACAC	667	39
BseAI	T/CCGGA	386	320
BseMII	CTCAG	646	60
BseXI	GCAGC	593	113
BslFI	GGGAC	572	134
BsmAI	GTCTC	656	50
BsmFI	GGGAC	572	134
BsmI	GAATGC	449	257
Bso31I	GGTCTC	657	49
Bsp120I	G/GGCC	598	108
Bsp13I	T/CCGGA	386	320
BspCNI	CTCAG	647	59
BspEI	T/CCGGA	386	320
BspMI	ACCTGC	593	113
BspMII	T/CCGGA	386	320
BspPI	GGATC	616	90
BspTNI	GGTCTC	657	49
BsrBI	CCG/CTC	617	89
BstHHI	GCG/C	368	338
BstMAI	GTCTC	656	50
BstV1I	GCAGC	593	113
BveI	ACCTGC	593	113
CfoI	GCG/C	368	338
Cfr9I	C/CCGGG	602	104
Eco31I	GGTCTC	657	49
EgeI	GGC/GCC	369	337
EheI	GGC/GCC	369	337
FaqI	GGGAC	572	134
FinI	GGGAC	587	119
FspBI	C/TAG	368	338
HhaI	GCG/C	368	338
Hin4II	CCTTC	595	111
Hin6I	G/CGC	370	336
HinP1I	G/CGC	370	336
HphI	GGTGA	555	151
HpyAV	CCTTC	584	122
HspAI	G/CGC	370	336
KasI	G/GCGCC	371	335
Kpn2I	T/CCGGA	386	320

Appendix 7. Restriction enzyme fragment lengths for the COI gene in round whitefish.

Enzyme	Restriction site	Fragment length (bp)		
MaeI	C/TAG	368	338	
MbiI	CCG/CTC	617	89	
MlsI	TGG/CCA	690	16	
MluNI	TGG/CCA	690	16	
Mly113I	GG/CGCC	370	336	
MroI	T/CCGGA	386	320	
MscI	TGG/CCA	690	16	
Msp20I	TGG/CCA	690	16	
Mva1269I	GAATGC	449	257	
NarI	GG/CGCC	370	336	
PctI	GAATGC	449	257	
PshBI	AT/TAAT	480	226	
PspAI	C/CCGGG	602	104	
PspOMI	G/GGCC	598	108	
SfoI	GGC/GCC	369	337	
SmaI	CCC/GGG	600	106	
SspD5I	GGTGA	555	151	
SspI	AAT/ATT	677	29	
TaqII	GACCGA	624	82	
UthSI	CC/CGGG	601	105	
VspI	AT/TAAT	480	226	
XmaCI	C/CCGGG	602	104	
XmaI	C/CCGGG	602	104	
XspI	C/TAG	368	338	
2 cuts:				
AciI	C/CAC	457	160	89
BccI	CCATC	301	205	200
BseYI	C/CCAGC	493	144	69
BspNCI	CCAGA	580	114	12
CviAII	C/ATG	435	155	116
FatI	CATG	437	153	116
Hin1II	CATG	432	158	116
Hsp92II	CATG	432	158	116
MroNI	G/CCGGC	368	332	6
NaeI	GCC/GGC	366	334	6
NgoMIV	G/CCGGC	368	332	6
NlaIII	CATG	432	158	116
PdiI	GCC/GGC	366	334	6
PsiI	TTA/TAA	539	135	32
SimI	GG/GTC	636	52	18
SsiI	C/CAC	457	160	89
TspDTI	ATGAA	364	206	136
TspGWI	ACGGA	413	207	86
3 cuts:				
AluI	AG/CT	275	182	155
BfuCI	GATC	476	107	80
				43

Appendix 7. Restriction enzyme fragment lengths for the COI gene in round whitefish.

Enzyme	Restriction site			Fragment length (bp)		
BseGI	GGATG	353	300	29	24	
Bsp143I	GATC	476	107	80	43	
BstF5I	GGATG	353	300	29	24	
BstKTI	GAT/C	472	107	84	43	
BstMBI	GATC	476	107	80	43	
ChaI	GATC	471	107	85	43	
DpnI	GA/TC	473	107	83	43	
DpnII	GATC	476	107	80	43	
FokI	GGATG	346	307	29	24	
Kzo9I	GATC	476	107	80	43	
MaiI	GA/TC	473	107	83	43	
MboI	GATC	476	107	80	43	
NdeII	GATC	476	107	80	43	
Sau3AI	GATC	476	107	80	43	
StsI	GGATG	345	308	29	24	
4 cuts:						
BceAI	ACGGC	306	165	142	78	15
Bcefl	ACGGC	306	165	142	78	15
MboII	GAAGA	298	280	110	15	3
MseI	T/TAA	407	167	59	52	21
Sth132I	CCCG	441	136	111	16	2
Tru1I	T/TAA	407	167	59	52	21
Tru9I	T/TAA	407	167	59	52	21
5 cuts:						
BshFI	GG/CC	295	162	110	81	42
BsiSI	C/CGG	367	216	54	51	12
BspANI	GG/CC	295	162	110	81	42
BsuRI	GG/CC	295	162	110	81	42
HaeIII	GG/CC	295	162	110	81	42
HpaII	C/CGG	367	216	54	51	12
HpaII	C/CGG	367	216	54	51	12
MspI	C/CGG	367	216	54	51	12
PalI	GG/CC	295	162	110	81	42
PhoI	GG/CC	295	162	110	81	42
Sth302II	CC/GG	366	216	54	52	12
6 cuts:						
Sse9I	AATT	241	197	128	50	39
TasI	AATT	241	197	128	50	39
Tsp509I	AATT	241	197	128	50	39
TspEI	AATT	241	197	128	50	39
7 cuts:						
MnlI	CCTC	211	127	114	77	64
					51	45
						17

Appendix 8. Restriction enzyme fragment lengths for the COI gene in *inconnu*.

Enzyme	Restriction site	Fragment length (bp)	
1 cut:			
Acc36I	ACCTGC	593	113
AccBSI	CCG/CTC	617	89
AclWI	GGATC	661	45
AfaI	GT/AC	552	154
AlwI	GGATC	661	45
ApaI	GGGCC/C	465	241
AssI	AGT/ACT	552	154
BbvI	GCAGC	593	113
BclII	T/GATCA	582	124
BfaI	C/TAG	578	128
BfiI	ACTGGG	514	192
BfuAI	ACCTGC	593	113
BinI	GGATC	661	45
BmrI	ACTGGG	514	192
BsbI	CAACAC	667	39
BscGI	CCCGT	415	291
BseII	ACTGG	510	196
BseNI	ACTGG	510	196
BseXI	GCAGC	593	113
Bsp120I	G/GGCC	469	237
BspMI	ACCTGC	593	113
BspPI	GGATC	661	45
BsrBI	CCG/CTC	617	89
BsrI	ACTGG	510	196
BsrSI	ACTGG	510	196
BstV1I	GCAGC	593	113
BveI	ACCTGC	593	113
Cfr9I	C/CCGGG	602	104
Csp6I	G/TAC	551	155
EciI	GGCGGA	660	46
EsaBC3I	TC/GA	403	303
FbaI	T/GATCA	582	124
FspBI	C/TAG	578	128
Ksp22I	T/GATCA	582	124
MaeI	C/TAG	578	128
MbiI	CCG/CTC	617	89
MroNI	G/CCGGC	368	338
Nael	GCC/GGC	366	340
NgoMIV	G/CCGGC	368	338
PdiI	GCC/GGC	366	340
PspAI	C/CCGGG	602	104
PspOMI	G/GGCC	469	237
RsaI	GT/AC	552	154
SapI	GCTCTTC	589	117
ScaI	AGT/ACT	552	154
SimI	GG/GTC	636	70
SmaI	CCC/GGG	600	106

Appendix 8. Restriction enzyme fragment lengths for the COI gene in *inconnu*.

Enzyme	Restriction site	Fragment length (bp)		
SspI	AAT/ATT	677	29	
TaqI	T/CGA	402	304	
TspGWI	ACGGA	413	293	
UthSI	CC/CGGG	601	105	
XmaCI	C/CCGGG	602	104	
XmaI	C/CCGGG	602	104	
XspI	C/TAG	578	128	
ZrmI	AGT/ACT	552	154	
2 cuts:				
AccIII	T/CCGGA	374	320	12
AluI	AG/CT	276	275	155
Aor13HI	T/CCGGA	374	320	12
AseI	AT/TAAT	262	226	218
BfuCI	GATC	528	123	55
BflI	T/CCGGA	374	320	12
BseAI	T/CCGGA	374	320	12
BseMII	CTCAG	621	60	25
BseRI	GAGGAG	454	209	43
Bsp13I	T/CCGGA	374	320	12
Bsp143I	GATC	528	123	55
BspCNI	CTCAG	622	59	25
BspEI	T/CCGGA	374	320	12
BspMII	T/CCGGA	374	320	12
BspNCI	CCAGA	340	252	114
Bst6I	CTCTTC	563	117	26
BstKTI	GAT/C	528	127	51
BstMBI	GATC	528	123	55
Chal	GATC	528	128	50
CstMI	AAGGAG	379	309	18
DpnI	GA/TC	528	126	52
DpnII	GATC	528	123	55
Eam1104I	CTCTTC	563	117	26
EarI	CTCTTC	563	117	26
FauI	CCCGC	379	270	57
FokI	GGATG	346	226	134
Kpn2I	T/CCGGA	374	320	12
Ksp632I	CTCTTC	563	117	26
Kzo9I	GATC	528	123	55
Mall	GA/TC	528	126	52
MboI	GATC	528	123	55
MboII	GAAGA	563	124	19
MroI	T/CCGGA	374	320	12
NdeII	GATC	528	123	55
PshBI	AT/TAAT	262	226	218
PsiI	TTA/TAA	476	135	95
Sau3AI	GATC	528	123	55
SmuI	CCCGC	379	270	57

Appendix 8. Restriction enzyme fragment lengths for the COI gene in *inconnu*.

Enzyme	Restriction site	Fragment length (bp)									
StsI	GGATG	345	226	135							
VspI	AT/TAAT	262	226	218							
3 cuts:											
BceAI	ACGGC	306	165	157	78						
BceFI	ACGGC	306	165	157	78						
BseGI	GGATG	345	226	127	8						
BstF5I	GGATG	345	226	127	8						
Sse9I	AATT	271	188	167	80						
TasI	AATT	271	188	167	80						
Tsp509I	AATT	271	188	167	80						
TspDTI	ATGAA	278	206	136	86						
TspEI	AATT	271	188	167	80						
4 cuts:											
CviAII	C/ATG	324	155	111	92	24					
FatI	CATG	324	153	113	92	24					
Hin1II	CATG	324	158	108	92	24					
Hin4II	CCTTC	264	158	139	127	18					
HpyAV	CCTTC	264	169	128	127	18					
Hsp92II	CATG	324	158	108	92	24					
NlaIII	CATG	324	158	108	92	24					
5 cuts:											
AciI	C/CGC	282	175	95	89	61	4				
BshFI	GG/CC	251	152	87	81	75	60				
BspANI	GG/CC	251	152	87	81	75	60				
BsuRI	GG/CC	251	152	87	81	75	60				
HaeIII	GG/CC	251	152	87	81	75	60				
PalI	GG/CC	251	152	87	81	75	60				
PhoI	GG/CC	251	152	87	81	75	60				
SsiI	C/CGC	282	175	95	89	61	4				
6 cuts:											
MseI	T/TAA	189	167	138	80	59	52	21			
Tru1I	T/TAA	189	167	138	80	59	52	21			
Tru9I	T/TAA	189	167	138	80	59	52	21			
7 cuts:											
8 cuts:											
9 cuts:											
BsiSI	C/CGG	216	155	116	57	54	51	28	12	11	6
HpaII	C/CGG	216	155	116	57	54	51	28	12	11	6
HpaII	C/CGG	216	155	116	57	54	51	28	12	11	6
MspI	C/CGG	216	155	116	57	54	51	28	12	11	6
Sth302II	CC/GG	216	155	116	57	54	52	28	12	10	6

Appendix 8. Restriction enzyme fragment lengths for the COI gene in *inconnu*.

Enzyme	Restriction site	Fragment length (bp)										
10 cuts:												
MnII	CCTC	211	130	114	77	50	25	25	23	20	17	14
Sth132I	CCCG	144	129	121	111	80	57	34	16	7	5	2

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